Supplementary Information:

Parallel paleogenomic transects reveal complex genetic history of early European farmers

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Supplementary note 1: Archaeological summary of the Neolithic and Chalcolithic periods in the region of today's Hungary

The Carpathian Basin (including the reagion of today's Hungary) played a prominent role in all prehistoric periods: it was the core territory of one cultural complex and, at the same time, the periphery of another, and it also acted as a mediating or contact zone. The archaeological record thus preserves evidence of contacts with diverse regions, whose vestiges can be found on settlements and in the cemeteries (grave inventories) as well.

The earliest farmers arrived in the Carpathian Basin from southeastern Europe ca. 6000–5800 BCE and they culturally belonged to the Körös-Çris (east) and Starčevo (west) archaeological formations [1, 2, 3, 4]. They probably encountered some hunter-gatherer groups in the Carpathian Basin, whose archaeological traces are still scarce [5], and bioarchaeological remains are almost unknown from Hungary. The farmer communities east (Alföld) and west (Transdanubia) of the Danube River developed in parallel, giving rise around 5600/5400 BCE to a number of cultural groups of the Linearband Ceramic (LBK) culture [6, 7, 8]. The LBK groups in eastern Hungarian Plain (ALPc) and in the western Hungarian Transdanubia (LBKT) developed diverse ceramic traditions. The western LBKT rapidly disseminated towards Central and Western Europe, whereas the ALPc, emerging in the northern parts of the Alföld, did not stretch out of the region of eastern Carpathian Basin, but developed internally. Around 5300 BCE the ALPc split into regional groups (Bükk, Tiszadob, Esztár, and Szakálhát) [7, 8, 9, 10]. In the form of a small colony, the north Balkanic early Vinča culture appeared in southwest Hungary between 5350-5100 BCE, connecting southeast Europe with the LBKT culture [11]. The further cultural development of the eastern and western parts of the Carpathian Basin remained mostly distinct in the local Late Neolithic (ca. 5000-4500 BCE), with the transition of the Szakálhát and late ALPc to the Tisza culture in the East; and the spread of the Sopot culture of southern origin [12, 13, 14, 15, 16, 17, 18], followed by the Lengyel cultural orbit, surviving until 4300 BCE to the western part of today's Hungary. The Lengyel culture spread over the Danube valley towards north and west, while some groups headed over the north-east Hungarian river valleys towards modern Poland [14, 19, 20, 21] Copper objects already appeared in the Late Neolithic communities, and some Lengyel culture settlements persisted in the following Early Chalcolithic (late Lengyel period in ca. 4500-4300 BCE), while the tell mounds of the Tisza culture were mostly replaced by flat single layer settlements of the first Chalcolithic Tiszapolgár culture in the east [22].

Intensive cultural contacts with southeastern Europe were documented in several phases over the more than two millennia, from the early 6th to the end of 5th millennium BCE, such as in the earliest Neolithic, the cultural influences of the north balkanic Vinča, the Croatian Sopot cultures or the southeastern connections of the Chalcolithic Lasinja and Baden complexes [23, 24, 25]. On the other hand two cultures have documented northwestern Central European connections from the investigated Hungarian series: the LBKT and the Lengyel culture. The question whether these trade and cultural connections were accompanied by movement of people, was debated for long in

the archaeological research of these periods, now it seems to have confirmed that the network was built out by mobile population groups.

In the last phase of the local Neolithic and at the beginning of the Copper Age (around ca. 4500-4000 BCE) significant changes took place in Central and southeastern Europe, both regarding settlement systems and subsistence strategies. These changes included the abandonment of steady, major settlements to a more scattered village structure, parallel with the increasing importance of animal husbandry, especially that of cattle.

In the local Middle Copper Age (ca. 4300-3800/3600 BCE), the (Balaton)-Lasinja-Ludanice-Bodrogkeresztúr period was followed by a period that can be considered to be transitional from several aspects ("Lasinja III", "proto-Cernavoda" and "Protoboleráz" can also be found in publications) [26, 27, 28]. Recently, the name "Protoboleráz" is generally accepted as a transitional "mixed" ceramic style lasted from ca. 3800-3600 BCE [29, 30, 31].

The Middle Copper Age's regional patterns seem to be homogenized by the changes in the Late Copper Age (ca. 3600–2850 BCE), when the Boleráz and Baden cultural complexes unified most of the territories of the Carpathian Basin for the first time of the region's prehistory. This period saw the introduction of several major innovations such as the wheel and various wheeled vehicles and the secondary exploitation of animals for their milk, wool and traction power (the latter was termed the Secondary Product Revolution by A. Sherratt) [32, 33, 34], as well as the appearance of wool sheep, the domestication of the horse, distinctive metalworking regions, the custom of raising burial mounds over the deceased, and burial steles [35, 36, 37]. These innovations and novelties were precipitated by radical social and economic changes. New innovations and inventions as well as new subsistence practices were only born or adopted in regions where environmental conditions and the available economic resources were conducive to this, and where there was a definite social demand for these innovations. Most of these innovations were initially linked to the cradle of civilisation, to Mesopotamia and Anatolia. More recent research has convincingly demonstrated the existence of three major centres of innovation: in addition to Mesopotamia and the Ancient Near East, there is evidence that the Maikop culture of the Caucasus and the Baden complex in Carpathian Basin were the two other cultural milieus, where new inventions and innovations regularly appeared [38]. The identification of the region(s) where the Copper Age innovations and inventions first appeared lends particular importance to the Carpathian Basin, where these innovations all appeared, and, moreover, it would seem that one of the centres of the invention of wheeled vehicles laid in this region, as suggested by the date of several more recent finds [39].

The presence of several archaeological cultures or cultural complexes (Coţofeni, Kostolac, Yamna, Baden) can be demonstrated in the Carpathian Basin during the Late Copper Age; several burial grounds and graves of these cultures are known, whose overall assessment and coverage varies. The Baden complex dominated the greater part of the Carpathian Basin: most of the period's burials can be assigned to this cultural complex, a conglomerate of various traditions and of various mortuary practices and customs, reflected also in the diversity of its mortuary symbolism.

The burials of the Baden complex reflect extremely diverse mortuary practices and they are therefore eminently suitable for examining similar phenomena and possible cultural connections over an extensive area. The complex's graves include both inhumation and cremation burials. Very often, a grave contained several burials, but skull burials and symbolic graves (the latter often empty or containing but a few artefacts) are also known. Some graves contained both human and animal burials. The deposition of the ashes in urns modelled on the human body represent a most singular mortuary rite. On many sites, complete or partial human remains were found dumped into a pit alongside "ritual animal burials" [40, 41, 42].

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Supplementary note 2: Description of archaeological sites

The following site descriptions pertain to newly reported samples, in particular from sites not covered in previous ancient DNA publications; see references from the main text for existing literature.

1. Sites in Hungary from the former German Research Foundation (DFG) project AL 287-10-1 (+Alsónyék site 11, Tiszaszőlős-Domaháza, and Törökszentmiklós road 4 site 3):

Alsónyék-Bátaszék, Mérnöki telep

In 2006-2009, an extended of the Starčevo culture, and features of the LBK and late Neolithic Lengyel cultures were unearthed at the Mérnöki telep subsite by the Institute of Archaeology, HAS (excavations directed by: A. Osztás and I. Zalai-Gaál). Out of the 1568 excavated features more than 400 belonged to the Starčevo culture (from the Linear B to the Spiraloid B phases), concentrated mainly in the southern part of the area. The majority of the features were pits, in various shapes and sizes. Besides pits, ditches and several types of ovens were excavated. A total of 25 burials belonged to the Starčevo occupation of the site. They were found either inside of ovens or in pits, in crouched position, without any trend in body orientation. Only one burial contained grave good [1]. Skeletons buried without grave goods, were radiocarbon dated in the CEZA laboratory in Mannheim, Germany and an absolute chronological framework for the development of the settlement was evaluated along with further radiocarbon measurements from the site [2].

Studied graves:

Feature 745 (BAM_4a)

An adult (35-45 years old) with indifferent osteological sex determination. He was genetically a male individual. The skeleton laid in crouched position, on its left side, without grave goods. The skeleton was radiocarbon dated to the Starčevo period: 5641-5547 cal BCE with 95.4 % CI (6677±27 BP, MAMS-11928).

Feature 1435 (BAM_13)

A ca. 8-9 years old child. He was genetically a male individual. The skeleton laid in crouched position, on the right side, without any grave goods.

The skeleton was radiocarbon dated to the Starčevo period: 5704-5556 cal BCE with 95.4 % CI (6704±34 BP, MAMS-11933).

Feature 1483 (BAM_17b)

A left-crouched skeleton of a ca. 7-8 years old child. He was genetically a male individual. The skeleton was radiocarbon dated to the Starčevo period: 5832-5667 cal BCE with 95.4 % CI (6857±31 BP, MAMS-11935).

Feature 1532 (BAM 25)

A ca. 20-30 years old individual with indifferent osteological sex determination [3]. He was genetically a male individual. The skeleton was presumably right-crouched in the grave.

The burial was radiocarbon dated to the Starčevo period: 5702-5536 cal BCE with 95.4 % CI (6695±40 BP, MAMS-11939).

Alsónyék-Elkerülő site no. 2. (5603/2)

Alsónyék-Elkerülő (also known as Alsónyék-Hosszú-dűlő, site 2) is a subsite of the Alsónyék site complex, which was a settlement and burial ground of the Sopot culture, dated to the turn of the 6-5th millennium BCE. It was excavated in 2008-2009 by J. Ódor from the Wosinsky Mór Museum, Szekszárd. Settlement features of the Sopot culture came to light in a small area, which included ten large, complex pits (with diameters of 2–7 m), a well, and short stretches of four more or less parallel ditches. According to the stratigraphy, most of the pits are earlier than the ditches. Traces of four houses, possibly belonging to the Sopot occupation, were also identified by geophysical survey. In sum, eighteen graves with the remains of 20 individuals were also excavated and identified by their associated material culture as belonging to the Sopot occupation.18 Sopot burials were sampled for the former DFG project of K. W. Alt, and three of them were further analyzed in this current genomic project. The occupation of the site was studied by a radiocarbon dating programme [4].

Studied graves:

Feature 220A (ALE 4)

Grave of an adult (35-45 years old) male individual (both osteologically and genetically).

The skeletons laid in left-crouched position in an oval-shaped double burial without grave goods (scattered pottery pieces only). The burial was radiocarbon dated to the Sopot period: 5016-4838 cal BCE with 95.4 % CI (6032±32 BP, MAMS-14814).

Feature 463 (ALE_14)

Grave of a ca. 6 years old child, genetically male.

Body laid in crouched position in a rectangular-shaped grave. A fragmented vessel and a flint blade were found in the grave. The human bones were radiocarbon dated to the Sopot period: 5030-4848 cal BCE with 95.4 % CI (6049 ± 29 BP, MAMS-14817).

Feature 464 (ALE_15)

Grave of an adult (40-45 years old) male individual. Body laid in left-crouched position in a rectangular-shaped grave. The grave had rich inventory of different types of vessels, stone axe, chipped stone tools, polished stone adze, and bone tool. The skeleton was radiocarbon dated to the Sopot period: 5208-4944 cal BCE with 95.4 % CI (6111 ± 36 BP, OxA-27578).

The anthropological determination was performed by Kitti Köhler (unpublished).

Alsónyék, Malomréti-dűlő, site T011.

In the central part of the main area of the Alsónyék complex investigated an LBK settlement was discovered. The features belonging to the LBK occupation were uncovered in subsites 10B, 11 and 5603. The location of houses could be determined by the long pits flanking presumed timber-framed constructions; postholes are very poorly preserved. Fifty house plans could be identified, most of them based on the remains of long pits.

Oross et al. presented 23 radiocarbon dates from 21 samples, interpreted within a formal chronological framework, for the LBK settlement at Alsónyék site 11. The LBK activity probably began in 5335–5280 cal BCE, probably lasted for 290–410 years, and probably ended in 5010–4915 cal BCE (all 68% probability).

Five LBK settlement burials were dug into house long pits. The mortuary practice was rather uniform, as all of the deceased were deposited into western long pits, closely associated with houses. The four carefully excavated bodies were left-crouched and oriented east—west, while the fifth was extremely disturbed [5].

The Copper Age part of the site has not been published or evaluated yet. It was excavated by the Ásatárs Ltd. (Zs. Gallina and P. Hornok) from 2006-2007.

Studied graves:

Feature 633 (GEN_100)

This is a Copper Age Balaton-Lasinja period grave of a genetically female individual. She laid straight in a circular pit on her right side, facing to the right, and had two typical Balaton-Lasinja type pots as grave goods.

Feature 1972 (GEN_18)

This LBKT grave was dug into one of the long pit of an LBK longhouse. A left-crouched skeleton of a 40–45-year-old male individual was found in the grave, who was a radiocarbon dated to 5309-5074 cal BCE with 95.4 % CI (6244±31 BP, SUERC-51459).

Bátaszék-Lajvérpuszta

Connecting to the Alsónyék site complex, a Lengyel grave group at Bátaszék-Lajvérpuszta is dated to the late Neolithic. It was excavated in 2009 by V. Majerik, from the National Heritage Protection Centre of the Hungarian National Museum, Department Pécs. The archaeological evaluation of the prehistoric findings is still ongoing (J. Ódor, personal communication). We sampled and studied in the former mtDNA project the 25 best preserved skeletons out of the 34 Neolithic burials, using the documentation of the excavation.

Studied graves:

Feature 35 (BAL_3)

Grave of an adult (maturus) male individual. Body was found in crouched position, lying on his left side in E–W orientation. The grave was assigned to the Lengyel culture considering grave goods, position of the grave and the general archeological context.

Feature 93 (BAL 25)

Grave of an adult (maturus) male individual. The skeleton was found in crouched position, lying on his back in SE–NW orientation without grave good. The burial was dated in this project to the Middle Neolithic LBKT period: 5208-4948 cal BCE with 95.4 % CI (6115±35 BP, Poz-82584).

The anthropological determination was performed by Kitti Köhler (unpublished).

Bölcske-Gyűrűsvölgy

On the M6 motorway track at Bölcske-Gyűrűsvölgy, 1.6 hectare surface was excavated in 2008 by the ELTE University (director of the excavation: K. Sebők). Altogether 257 features from four archaeological periods (LBKT, Bronze Age, La Téne B-C, and Árpád Age) were uncovered on the site. LBKT features from the *Zseliz/Želiezovce* phase with typical red-yellow painted pottery and Tisza import ware were reported from the site. Five LBKT graves came to light: from a clay pit (feature no. 36) a triple grave, and from another pit a double burial. Two grave goods are mentioned: a large globular pot and a vessel with *Notenkopf* decoration [6, 7].

Studied grave:

Feature 41/STR 55 (BOVO_1b)

Crouched, damaged skeleton of a juvenile individual, laid in a pit complex containing three burials. The genetic analyses determined the sex of the skeleton to be a female. The grave had no grave good. However it was dated to the LBKT period, based on the archaeological observations of K. Sebők.

Budakeszi, Szőlőskert-Tangazdaság

At the site Budakeszi, Szőlőskert-Tangazdaság Neolithic and Early Bronze Age settlements were unearthed in 2006 by the Directorate of Pest County Museums (director of the excavation: A. Czene). On a surface of one hectare, 632 features were excavated; most of them belonged to the *Notenkopf* and *Zselíz/Želiezovce* phase of the LBKT. Several clay pits and storage pits were found, with large quantity of animal bones. A segment of a building construction was indicated by a sequence of postholes. On the area of the LBKT settlement, 15 burials were uncovered. Most of them laid in pits, in crouched positions without any grave good. One of them had a rich inventory of vessels (six pieces, partly with *Notenkopf* pattern) [8, 9]. Four skeletons were dated in the CEZA laboratory in Mannheim. The radiocarbon results date the burials to the *Notenkopf* and *Zselíz/Želiezovce* phase of the LBKT [10]. Anthropological analyses were performed by K. Köhler [11].

Studied graves:

Feature 290 (BUD_4a)

Adult male (25-35 years old). The genetic analyses supported the osteological sex determination. The skeleton was found in crouched position, lying on his right side. A large grinding (?) stone was found beside the legs.

Feature 389 (BUD_9a)

Subadult probably male (17-19 years old). The genetic analyses contradicted the osteological sex determination, she was genetically a female. The skeleton was found in crouched position, lying on its left side.

Cegléd 4/1, Váróczi-Hodula-dűlő

The site is situated in central Hungary, about 80 km East from Budapest and about 17 km West from River Tisza. Cegléd 4/1 was excavated by the Directorate of the Museums of Pest County (today Ferenczy Museum) in 2003-2004. There are three different settlements on the site from three different periods, a Middle Neolithic settlement, a Sarmatian settlement from the Roman Age, and an early medieval village from the Árpád Age. The Neolithic features were different kind of pits (refuse and borrow pits), a part of a double ditch-system with "V"-shaped cross-section, and 15 graves scattered on the whole territory of the settlement. The pits contained a huge amount of ceramic finds from the early ALPc-Szakálhát culture, and some bone, stone and clay implements. A bowl with *Notenkopf* decoration was found, possibly an import from Transdanubia, western Hungary. Every burial followed the typical burial rites of the Szakálhát culture, they were inhumation burials, lay on their left side in crouched position, with orientation SE-NW. Six burials contained grave goods: there was a Spondylus disc in the grave of two children, one piece of big Spondylus bead in three graves, and 15 tiny Spondylus beads in one of the graves. The skeletons

laid in shallow pits, six of them were rectangular, six of them were oval, and one was irregular. Two skeletons laid high in the brown humus, the shape of their grave pits could not have been identified [12].

Studied graves:

Feature 451 (CEG03b)

Grave of an adult male (30-40 years old) individual. The genetic analyses supported the osteological sex determination.

The body was found in crouched position, lying on his back, without any grave goods.

Feature 546 (CEG07b)

Grave of an adult (maturus) male (40-50 years old) individual. The genetic analyses supported the osteological sex determination.

Feature 560 (CEG08b)

Grave of an infans II child (ca. 8 years old). She was genetically a female. The body was found in crouched position, laid on the left side, in SE-NW direction. As a grave good, one pierced Spondylus disk can be mentioned, while fragments of two large vessels in Szakálhát pottery style came to light from the upper layer of the grave pit.

The anthropological evaluation of the human remains was carried out by Kitti Köhler (unpublished).

Csabdi-Télizöldes

At the site Csabdi-Télizöldes, a settlement and a cemetery from the early phase of the Lengyel culture were excavated by J. Antoni between 1978 and 1986. According to the preliminary publication, 32 graves were found among the settlement features altogether [13], but the archaeological material is still unpublished. The archaeological and anthropological material is stored in the Szent István Király Museum (King St. Stephen Museum, Székesfehérvár). The anthropological analyses were carried out by K. Köhler [14].

Studied graves:

Grave 18. (79.VII.18, CSAT_19a)

Grave of an adult male (30-36 years old) individual. The genetic analyses supported the osteological sex determination. Body was found in crouched position, lying on his right side in SW-NE direction. Following grave goods were found in the burial: four different types of vessels, one piece of (Spondylus?) bead.

Grave 25. (CSAT_25a)

An adult (maturus) male (40-60 years old). The genetic analyses supported the osteological sex determination. It was a disturbed grave, the fragmented skeleton was found in uncertain position, without any grave good. The grave was dated in the frame of this project to the Lengyel period: 4826-4602 cal BCE with 95.4 % CI (5850±40 BP, Poz-82582).

Ebes, Sajtgyár (Cheese Factory), site 19.

Although this excavation was situated on the other bank of the Zsong brook, the two settlements seems to be archeologicaly contemporary, and - with all probability - the two settlement parts were connected to each other. The excavation took place in the autumn of 2010. The most intensive and significant timeperiod of this rescue excavation was the late phase of the Middle Neolithic with some huge, primary claypit (functioned secondary as waste pits and in some cases as burial places). The intensity of this Middle Neolithic settlement was increased in northern direction, so it is sure that in 2010 excavated area is a part of the earlier observed bigger ALPc settlement under the present-day Curver factory [15, 16].

On the approx. 1/3 hectares big researched area alltogether four graves came to light from the ALPc-Esztár group. Three out of the four graves were unearthed from the uppermost level of big waste pits, and only one has got own isolated gravepit. All excavated graves were without discernible gravegoods.

Studied grave:

Feature 56/ STR 99 (EBSA_2a)

This grave was located in the uppermost part of the pit Feature 55/STR 98. A badly preserved skeleton (adult, female based on the genetic sex determination) was buried on its left side in a strongly crouched position. Orientation: SE-NW 154°. No grave goods were observed.

The anthropological evaluation of the remains was carried out by Zsuzsanna K. Zoffmann (unpublished).

Ebes, Zsong-völgy

The first excavation took place in 2003 on this huge multiperiod site. Then there were rescue excavations here in 2004, 2007 and lastly in 2015.

Beside other periods (Middle Iron Age, Roman Imperial period, Late Avar period, Árpád Age) there was unearthed here a huge extensive settlement of the ALPc-Esztár group, which was connected closely to the riverbank [17]. The settlement consists of little and bigger amorphous clay

and storage pits (with secondary, mainly waste storage function). Between the pits some ESE-WNW oriented inhumation graves in contracted position were came to light. Most of them were in independent, but shalow gravepits with rounded ends, but some human remains were found in the filling of the waste pits [18].

Studied grave:

Feature 1412/ STR 2989 (EBVO_5a)

Burial of an adult (maturus, 43-60 years old) male. The genetic analyses supported the osteological sex determination. The fragmented skeletal remains were found in the upper part of a shallow amorphous waste pit (Obj.1407/Str.2982).

The basic anthropological evaluation of the prehistoric materials was carried out by Zsuzsanna K. Zoffmann (unpublished).

Enese elkerülő, Kóny, Proletár-dűlő, M85 site II

Altogether, a surface of 18,000 m² was excavated between 2008 and 2009 on the site Enese elkerülő, Kóny, Proletár-dűlő II, by K. Varga from the Hungarian Field Service for cultural Heritage. Among the 941 archaeological features some could be dated to the LBKT and Lengyel and Balaton-Lasinja periods.

Two LBKT graves included grave goods. One skeleton was found on the left side, in a crouched position (grave 55) [19]. Two children without grave goods were laid in the grave 612. Our radiocarbon dating assigned this grave to the late LBKT period. One grave belonged to the Middle Copper Age based on its radiocarbon date (grave 223) and another had a vessel assigning a third grave to Lengyel III culture (grave 826) [20].

Studied graves:

Feature 223/ SNR 233 (KON 2)

Grave of an adult (maturus, 44-48 years old) female individual. The genetic analyses supported the osteological sex determination.

The skeleton was dated to the Balaton-Lasinja period: 4333-4072 cal BCE with 94.5% CI (5380±30 BP, Beta-310033).

Feature 286/ SNR 300 (KON 3)

Grave of a juvenile female (22-23 years old). The genetic analyses supported the osteological sex determination. The skeleton laid on its back, with legs flexed under the body. An LBKT type little cup, a bowl and a stone tool were her grave goods [19].

The basic anthropological evaluation of the prehistoric materials was carried out by Gábor Tóth (unpublished).

Fajsz-Garadomb

The site Fajsz-Garadomb was investigated in 2006-2008, as part of a cooperation of the Institute of Archaeology (HAS) and the University of Tübingen. The excavations were directed by E. Bánffy and J. Petrasch. The archaeological records have not been evaluated yet. LBK and Sopot settlement layers were found over the occupation traces of the Körös culture. There are four inhumation graves at Fajsz-Garadomb. Two are supine burials, and in one further case the human remains were carefully deposited in a secondary position. The genetically analyzed graves were dated by Sopot II type pottery and stratigraphic observations (unpublished data).

Studied graves:

Feature 156 (FAGA_2)

Burial of an adult female (25-30 years old). The genetic analyses supported the osteological sex determination. She was buried with grave goods, and also the stratigraphic position of the feature dated the grave to the Sopot period of the site.

The grave was dated in the frame of this project to the Sopot period: 5195-4842 cal BCE with 94.5% CI (6060±40 BP, Poz-83633).

Feature 165 (FAGA_1)

Grave of an adult (35-45 years old) male individual. The genetic analyses supported the osteological sex determination. He was buried without grave goods, but the stratigraphic position of this grave connects it to other Sopot period features of the site.

Felsőörs-Bárókert

In 2008, a large and multilayer Neolithic settlement was unearthed on a rescue excavation by J. Regenye from the Laczkó Dezső Museum (Veszprém). The area was settled from the second half of the sixth millennium BCE through the fifth millennium BCE. Five graves were unearthed from the second phase of the Lengyel culture, which have not been published yet [21].

Studied graves:

Feature 100 (FEB 3)

A genetically a male individual was buried here. The grave has neither been evaluated archaeologically nor anthropologically.

Hajdúnánás-Eszlári út

The Hajdúnánás-Eszlári út site (Hajdú-Bihar County, Hungary) was excavated in 2004 and 2005 by P. Raczky and A. Anders of the Institute of Archaeological Sciences, Eötvös Loránd University (Budapest) and by E. Gyöngyvér Nagy of the Hajdú-Bihar County Museum (Debrecen).

An area of approximately one hectare was uncovered along the planned track of the M3 Motorway. The small Middle Neolithic site yielded large pits and 57 inhumation graves as well as traces of a multiple ditch system. The pottery finds are characteristic of the Tiszadob–Bükk and Esztár ceramic style groups of the late phase of the ALPc. The burials formed smaller clusters of three to six graves. Some graves were dug into the upper part of large pits, while others lay on the prehistoric humus level. The deceased were buried according to the same rite: they were crouched on the left side and had a west to east orientation. Grave goods were recovered from the burials of four females and one child: a pair of vessels (in two burials) and Spondylus beads were deposited beside the deceased [22].

The 10 (unpublished) radiocarbon dates made on human and animal bones from the settlement span the time interval between 5300 and 5000 cal BC.

Studied graves:

Feature 50/ STR 93 (HAJE 1)

Grave of an adult (27-33 years old), osteologically and genetically male individual.

Feature 80/ STR 147 (HAJE_7)

Grave of a maturus (40-46 years old), osteologically and genetically male individual.

The skeleton was radiocarbon dated in the frame of this study to the ALPc period: 5302-5057 cal BCE with 94.5% CI (6220±40 BP, Poz-83631).

Feature 108/ STR 197 (HAJE 10)

A maturus (40-49 years old), osteologically and genetically male individual.

The skeleton was radiocarbon dated in the frame of this study to the ALPc period: 5221-5000 cal BCE with 94.5% CI (6170±40 BP, Poz-83632).

Hejőkürt-Lidl logisztikai központ

In 2005, a flat settlement of the ALPc-Tiszadob culture culture (some ceramics from the ALPc-Szakálhát group were also found) was excavated by J. Koós from the Herman Ottó Museum (Miskolc). Neolithic burials were found within the ALPc settlement, and also traces of other periods (Chalcolithic) came to light. The archaeological material is still under evaluation. The anthropological analyses are published [23].

Studied graves:

Feature 8/SNR 2317 (HELI_2)

Grave of a subadult (18-20 years old), both osteologically and genetically male individual.

Feature 153/ SNR 775 (HELI_11)

Burial of an adultus-maturus (30-60 years old), both osteologically and genetically male individual. The grave was dated in the frame of this project to the classic-late ALPc period: 5209-4912 cal BCE with 94.5% CI (6100±40 BP, Poz-88115).

Hódmezővásárhely-Gorzsa-Czukor major

In 1978-1996, a tell settlement from Late Neolithic Tisza culture was excavated by F. Horváth (Móra Ferenc Museum, Szeged). The activity on the site continued after the Neolithic through the Copper, Bronze and Iron Ages into the Sarmatian period. Neolithic burials were found in that parts of the occupation that were currently not used as a residential area [24, 25, 26]. Radiocarbon dates are available from the site [27]. The Tisza culture graves are still unpublished.

Studied graves:

Grave 4 (Gorzsa4)

Grave of an osteologically and genetically female adult individual.

Grave 18 (Gorzsa18)

Grave of an adult, osteologically and genetically male individual.

The anthropological re-evaluation of the human remain was carried out by Masson, M. [28, 29].

Hódmezővásárhely-Kökénydomb, Vörös tanya

The tell settlement of the Tisza culture was excavated in several campaigns between 1929-1940 and in 1985 by János Banner, József Korek, István Foltinyi and Ferenc Horváth. The extension of the researched area totaled 2.5 hectares. Grave 1 of the 1940 campaign came to light in the so called "Vörös-tanya" subsite of the Kökénydomb tell [30, 31]. The crouched skeleton was found in a refuse Pit "A", which yielded an antler harpoon and large amount of diagnostic Tisza-culture pottery.

Studied grave:

Grave 1. [1940] Inv. Nr. 159 (KÖKE_3)

Grave of an adult (osteologically and genetically) male individual [32]. The anthropological re-evaluation of the human remain was carried out by Masson, M. [29].

Keszthely-Fenékpuszta Pusztaszentegyházi-dűlő

In 2000, two pits of the Balaton-Lasinja culture were unearthed in Keszthely-Fenékpuszta, with several human skeletal remains, by Róbert Müller from the Balaton Museum in Keszthely. In feature 45, seven sculls were uncovered besides other scattered human bones. In feature 46, along the pit wall, the pectoral bones of a child, lying in anatomical order, came to light. In the fill of the pit, several other human bones were found as stray finds. The osteological analysis, carried out by Zs. Zoffmann, identified the bones of 14 children, two men, and three women [33]. Based on the find context and the evaluation of the comparable finds of the Balaton-Lasinja culture, features 45 and 46 can be interpreted as records for a secondary mortuary practice including manipulation of human skulls and postcranial skeletons [34].

Studied grave:

Feature 45. Inventory number 2000.01.02. (KEFP_2)

Skull of a 1.5-2 years old child, who was genetically determined as female.

Lánycsók – Csata-alja

The site lying in the southern Transdanubia was found and excavated in 2008, as part of the rescue excavations before constructing the motorway M6. The two hectares excavated by Vajda-Kiss from the Janus Pannonius Museum in Pécs, contained 433 Starčevo, and Balaton-Lasinja features, among later ones [35]. According to radiocarbon dating of the sampled specimens in the CEZA laboratory in Mannheim, three burials came from the Starčevo, and one from the Balaton-Lasinja period [36].

Studied grave:

Feature 221/SNR 383 (M6-116.12)

Skeleton of an osteologically and genetically female subadult (17-19 years old) individual. The body was found in stretched position, lying on her back, without any grave goods.

The skeleton was radiocarbon dated to the Balaton-Lasinja period: 4232-4046 cal BCE with 94.5% CI (5300±23 BP, MAMS-14132) [36].

Anthropological analyses were performed by Brigitta Ősz (unpublished).

Lánycsók-Gata-Csatola

The excavation at Lánycsók-Gata-Csatola was led by V. Voicsek from the Hungarian Field Service for cultural Heritage, Department Pécs. Remains of several archaeological periods were excavated in 2008: prehistory (Early Neolithic, Copper Age, Middle and Late Bronze Age, and Celtic period), Migration period (Avar and Conquest period graves) and Middle Ages.

Overall, 545 objects were unearthed on a surface of 3.5 hectares. About 30 Starčevo objects (pits, pit complexes, ovens, postholes, ditches) were found as well. Typical Starčevo pottery, for example low pedestals with applied barbotine and nail impressed ribs decorated shards, and clay bobs came to light from the pits. Out of 143 graves, five belonged to the Starčevo culture. These burials were found in pits, on the west part of the excavated area, in crouched positions [37].

Studied graves:

Feature 1661 (LGCS 1)

Burial of an adult (35-44 years old) male (both osteologically and genetically) individual. The skeleton laid in crouched position on his prone, his arms were stretched along, and the legs were bent under the body. The grave had no grave goods, but it was dated to the Starčevo period, based on the archaeological context.

Mezőkövesd-Mocsolyás

Excavation was led by J. Koós in 1993-1994. There was a flat settlement at Mezőkövesd-Mocsolyás from the earliest period of the ALPc- Szatmár group, with longhouses and 25 burials among the settlement features. Five houses was burned down, and could be observed and reconstructed in details. Absolute chronological data is available for the site, but no human remains were radiocarbon dated [38, 39, 40].

Studied graves:

Feature 8/ STR 192 (MEMO 7a)

Grave of an adult female (37-43 years old). The genetic analyses support the osteological sex determination. The body was found in crouched position, lying on her left side in SE-NW orientation. Grave goods: different types of Spondylus beads.

Feature 2/STR 103 (MEMO 2b)

Grave of an adult (maturus) male (35-55 years old) individual. The genetic analyses contradict the osteological sex determination, she was genetically a female.

The body was found in crouched position, lying on his back. Before the burial, the body was cut in half between the rib cage and hips and the two body parts were placed in two adjacent pit (upper part: pit 75/a, lower part: pit 103/a). There were no grave goods in the burial.

Feature 25/ STR 448 (MEMO_24b)

Grave of an adult male (34-40 years old) individual. The genetic analyses support the osteological sex determination. The body was found in crouched position, lying on his left side in SE-NW orientation. There were no grave goods in the burial.

Polgár-Piócási-dűlő

The salvage excavation took place in 2006–2007, preceding a major investment project and was led by the Déri Museum (Debrecen), during which associated settlement features of a Middle Neolithic ALPc were uncovered in an area called Piócási-dűlő on the southeastern outskirts of Polgár. The features of the ALPc settlement are dated in two periods. The cluster of multi-functional pits yielding a rich assortment of finds, the handful of post-holes and an unusual ritual well found in the southern part of the investigated area formed one unit from the earliest phase of the Middle Neolithic (ALPc I. phase). The settlement's other occupation can be assigned to the late phase of the Middle Neolithic (ALPc IV. phase). Five house plans representing the remains of timber-framed buildings outlined a distinct area with three multi-functional pits.

Associated with the above features were a few burials, which expressed the community's ritual beliefs. Some members of the community played a role in the settlement's life even after their death. The key to the selection of the individuals and their symbolic presence lies in the period's ritual memory. The eight graves contained the burials of five adults, two children and an infant, all dating from the late period. The adults were laid on their left side in a strongly crouched position; one burial contained a small vessel (Feature 32), and the remains of red ochre were identified in two other burials (Features 17 and 108). Four of the adult burials had been deposited in regular grave pits, while the fifth was found on the floor of a round pit. Since the formalized placement of the body could be noted in this case too, it can be regarded as a regular burial despite its unusual location [41, 42, 43].

Finds from two pits were radiocarbon-dated, but there has not been published any absolute chronological data of the human remains yet.

Studied grave:

Feature 108/ STR 173 (POPI_5)

Grave of a maturus (50-56 years old) female individual (based on the osteological determination). The genetic data contradict to this result and define the skeleton as to be genetically male. The skeleton was crouched on its left side, in SE-NW orientation. No grave goods except for some remains of red ochre were found in the grave.

The anthropological evaluation of the remains was carried out by Zsuzsanna K. Zoffmann (unpublished).

Pusztataskony-Ledence 1 (formerly 1 & 2)

The site was excavated between 2009-2011, by the Institute of Archaeological Sciences of the Eötvös Loránd University (excavation team: K. Sebők, G. Szabó, A. Füzesi, M. F. Tóth, Á. Király, F. Lamm, A. Kalli, M. Szilágyi, Zs. Masek). As it is positioned on a high bank of a former branch of the river Tisza, despite its size the site is extremely dense at some points, containing materials from several archaeological periods. Sporadic traces of a small settlement from the ALPc III-IV periods were observed here, as well as remains of an extended settlement of the ALPc-Szakálhát culture with altogether 13 burials with a special ceramic-covered burial of a child amongst them; traces of a large settlement of the Tisza Culture with 14 burials and strong Lengyel influence; a small cemetery of an Early Copper Age Tiszapolgár culture community with 7 burials altogether, completed perhaps by a nearby burial of a very special person with a Bodrogkeresztúr type vessel amongst her grave goods; remains of extensive earthworks and settlements of multiple periods from the Late Bronze Age Tumulus and Gáva cultures, with partial human remains in the pits and a child burial in a well; three 'mass graves' (collective secondary burials) from the Early Iron Age (Ha B2/B3); traces of a small Scythian settlement (7-6th century BCE) with three pit burials, and those of a Sarmatian Age settlement (3–4th c. AD) with a lonely burial; a Gepid cemetery of altogether 13 burials; a lonely burial from the early Avar period, and two more from the late Avar period; remains of a 18–19th century hamlet and irrigation canals. About 11 shallower, mostly crouched burials without any grave good must be added to some of the prehistoric periods — by their relative positions perhaps to the Early Copper Age. Radiocarbon data, evaluated by the Poznan Radiocarbon Laboratory, are available for the Tisza culture (4618-4502 cal BCE, 4530-4448 cal BCE) and for one of the Iron Age collective burials (821-796 cal BCE) [7, 44-48].

Studied graves:

Feature 130/ STR 150 (PULE1.9)

Grave of an adult individual, with indifferent osteological sex determination. He was genetically male. The burial was furnished with Tiszapolgár type pottery.

Feature 135/ STR 155 (PULE1.10)

A Tiszapolgár period grave of a subadult male (16-18 years old) individual based on the osteological analyses, who is genetically also a male. The body laid in crouched position and was accompanied by vessels as grave goods.

Feature 192/ STR 236 (PULE1.13)

Grave of an adult male individual, whose sex was also supported by the genetic analyses. The burial was richly furnished with Tiszapolgár type pottery.

Feature 269/ STR 335 (PULE1.18)

An ALPc-Szakálhát period grave with vessels as grave goods. The adult female (30-36 years old) individual was found in crouched position. Her sex was also supported by the genetic analyses.

Feature 319/ STR 396 (PULE1.22)

Burial of an adult male individual, whose sex was supported by the genetic analyses. The body laid in crouched position and was accompanied by Tiszapolgár type vessels as grave goods.

Feature 328/ STR 407 (PULE1.23)

Grave of an adult (matures) female (45-51 years old), the sex was also ascertained by the genetic analyses. The burial was assigned to the ALPc-Szakálhát culture based on the archaeological context.

Feature 365/ STR 460 (PULE1.24)

Richly furnished Tisza period grave of an adult (maturus, 49-55 years old) individual. Osteological determination of this individual was male but genetically she was a female.

Feature 419/ STR 537 (PULE1.26)

Richly furnished grave from the Tisza period. An adult (30-36 years old) osteologically male individual, who was genetically a female.

The anthropological evaluation of the human remains was carried out by Zsuzsanna K. Zoffmann (unpublished).

Szederkény-Kukorica-dűlő

The site Szederkény was unearthed between 2005 and 2008 by G. Kovaliczky from the Janus Pannonius Museum, Pécs. The excavated area totalled over 12.5 hectares, from which the Neolithic settlement covered nearly 9 hectares. In addition to the Neolithic settlement features, finds and features of later ages such as the Copper Age (Balaton-Lasinja, Baden), the Bronze Age (Encrusted Pottery, Urnfield), the La Tène and the late Roman period were unearthed alongside a late Roman and early Migration period cemetery. The archaeological material of the three excavated Neolithic house groups could be assigned to the early Vinča and early Sopot cultures. Remains of 66 Neolithic houses have been identified so far, and 50 graves could be dated to this period [49]. Only 11 early Vinča/Sopot burials were involved in the German-Hungarian DFG project (AL 287-10-1), because at the time of the sampling, the chronology of the rest of graves was uncertain. The five radiocarbon dates that were measured in the CEZA laboratory of Mannheim fall between 5360-5165 cal BCE on the two-sigma confidence level [50].

Studied graves:

Feature 119 (SEKU_1)

Grave of a genetically male individual.

The skeleton was radiocarbon dated: 5202-4851 cal BCE with 94.5% CI (6079±33 BP, MAMS-14808).

Feature 2398 (SEKU_6)

Grave of a genetically female individual.

The skeleton was radiocarbon dated: 5321-5081 cal BCE with 94.5% CI (6267±33 BP, MAMS14809).

Feature 2491 (SEKU_10)

Grave of an adult male (30-40 years old) individual, who was genetically also a male.

The skeleton was radiocarbon dated: 5320-5080 cal BCE with 94.5% CI (6264±34 BP, OxA-29050).

The basic anthropological evaluation of feature 2491 was carried out by Kitti Köhler (unpublished).

Szemely-Hegyes

The site was excavated on the Baranya county part of the M6 motorway track in 2006-2007. On a surface of over four hectares ca. 1400 archaeological features were documented from the *Keszthely* and *Notenkopf* phase of the LBKT, Sopot culture, and Copper Age Balaton-Lasinja and *Furchenstich* periods (T. Paluch, K. Somogyi personal comm.) [51]. Houses, pits, ditches, and ovens were uncovered on the LBKT settlement. From the ten Neolithic burials, six were radiocarbon dated in the Bioanalytic laboratory in Miami. Two graves gave late LBKT and four Vinča-Sopot dates [36]. The population composition of the community lived in Szemely is especially interesting, because it is located in the contact zone of the LBKT, Vinča, and Sopot cultures' territories [52].

Studied graves:

Feature 1003/SNR 598 (SZEH 5a)

Grave of a ca. 15-20 years old juvenile with indifferent osteological sex, who genetically proved to be a male. The skeleton was radiocarbon dated to the Sopot period: 4904–4709 cal BCE with 94.5% CI (5920±40 BP, Beta - 310039).

Feature 1085/SNR 827 (SZEH_7b)

Grave of a ca. 0.5 year old child, genetically female.

The skeleton was radiocarbon dated to the Sopot period: 4930–4715 cal BCE with 94.5% CI (5930±40 BP, Beta-310040).

The anthropological evaluation of the human remains was carried out by Kitti Köhler (unpublished).

Tiszadob, Ó-Kenéz

At Tiszadob, Ó-Kenéz a flat settlement of the ALPc-Tiszadob group with burials in adjoining living spaces was excavated in 2006-2007. 22 features with human remains were uncovered. The burials were found in loose clusters inside the trench that "divided" the Neolithic settlement. The majority of skeletons laid in a larger pit complex or in one of 2-3 pits, dug into one another. Seven skeletons were found in independent graves (234, 315, 328, 428, 496, 510.01, 519, and 523). The burials had a SE-NW orientation with smaller divergences. The majority of the skeletons had a SE-NW with the dead crouched on their left sides [53].

Studied graves:

Grave 251-01 (2011.6.12, TISO 1)

Grave of an 8-10 years old child, who is genetically male.

Grave 328 (2011.6.21, TISO_3)

Grave of an adult female (both osteologically and genetically). This individual was buried with her face turned downwards

Grave 472 (TISO_11)

Grave of an adult male (both osteologically and genetically). Remains of red ochre were found indicative of painting of the extremities.

Grave 478-01 (2011.6.8, TISO_13)

Grave of an adult male (both osteologically and genetically).

The skeleton was radiocarbon dated in the frame of this project to the ALPc period: 5208–4942 cal BCE with 94.5% CI (6110±40 BP, Poz-83630).

The anthropological evaluation of the human remains was carried out by László Szathmáry (unpublished).

Tiszaszőlős-Domaháza

In 2003 a small Körös culture settlement, extending no wider than 40 by 20 m, was found at Tiszaszőlős-Domaháza on the southbank section of an old oxbow of the Tisza river [54]. This site was found as a result of a deliberate search aiming to find new Körös sites north of their previously hypothesized northern border (along the line of Kunhegyes and Berettyóújfalu), which was believed to have separated the hunter-gatherers from the early farmers in the Early Neolithic. The research at the site was led by L. Domboróczki from the István Dobó Castle Museum, Eger. Ultimately two pits, a house plan and several graves were unearthed here from the time of the Körös culture.

The largest feature containing Körös finds was Pit 6. It measured 9 x 7.5 m and similarly to the site of the house, was directed NW–SE along its longer axis. It yielded large amount of ceramic sherds and, similarly to the site of the house, also contained large quantities of mussel shell, which here, however, constituted continuous layers within the fill. Pit 6 was almost 2 m deep and was uncovered in 15 artificial layers, each 10–20 cm in depth. Find material from the Körös culture was present in all layers. While in the lower layers it was exclusively the Körös finds that dominated, in the upper layers Middle Neolithic Szatmár Group finds also appeared, marking the beginning of the Alföld Linear Pottery Culture (ALPc).

Remains of at least seven human individuals were found at the site: two of them were found as complete skeletons laid in crouched position, two others occurred as more-or-less complete skeletons identifiable by their dispersed body parts, and three other human bodies were represented only by single bones. Out of these six individuals four belonged to graves.

Studied graves:

Grave 2-3 (TIDO2a)

Skeletal remains of a 31–37 years old male. The body, separated into two or three parts probably by later disturbance, was found in the lower (10–13th) layers of Pit 6. Originally it was registered as remains of two graves, but now assigned as Grave 2. The skeleton was radiocarbon dated to the Körös culture: 5736-5547 cal BCE (6740±60 BP, deb-11804).

Grave 4 (KO1, previously published [55])

Skull of a 15–17 years old (female) child that was found in the lower layers in Pit 6. Despite its clear Körös context and radiocarbon date, the skull genetically aligns with Mesolithic populations [55].

Grave 5 (TIDO3a)

A jaw of a 23–59 years old male found in Pit 6. Though it is not radiocarbon dated the Körös context is plausible.

Tolna-Mözs TO26

In 2008–2009, the Institute of Archaeology of the HAS led a large-scale excavation in Tolna-Mözs (Tolna County), along the track of the M6 motorway (the excavation was directed by Ferenc Redő and István Koós). LBKT, *Furchenstich*, Hallstatt, Celtic, Avar and Middle Age features came to light. Based on the excavation record, K. Oross and T. Marton could reconstruct 47 LBK houses, from postholes and long pits along the one-time longitudinal walls of the buildings. Combined with further evidence of a large-scale geomagnetic survey, a total of ca. 150 buildings can be suggested. The settlement was inhabited at least from the early *Bicske/Biňa* phase to the *Notenkopf* phase of the LBKT. The archaeological finds of the houses show a sequence of occupation in different pottery determined phases: the early house group contained ceramic material resembling Vinča and late Starčevo pottery types beside of early LBKT characteristics, whereas the later northern house group contained early LBKT and *Notenkopf* ceramic types. An LBKT grave was uncovered in the western long pit (Feature 1649) flanking house H35. Based on the archaeological context and the radiocarbon date, the grave could be considerably younger than the houses of the same, southern settlement part. The second radiocarbon dated skeleton was found in a crouched position, without any grave good [19, 56].

Studied grave:

Feature 1649 (TOLM 4)

Grave of an adult male individual (ca. 35-45 years old) in left-crouched position.

The skeleton was radiocarbon dated to the LBKT period: 5301-5076 cal BCE with 94.5% CI (6233±23 BP, MAMS-14145) [10].

The anthropological evaluation of the human remains was carried out by Kitti Köhler (unpublished).

Tolna-Mözs-Fehérvize-dűlő, TO03

In 2008, the Institute of Archaeology HAS excavated this subsite of the Tolna-Mözs complex. From the excavated 639 archaeological features, 141 could be assigned to the Balaton-Lasinja culture, by the excavator Tünde Horváth. Besides the Copper Age Lasinja, Early Bronze Age, Late Iron Age and Migration period features came to light.

According to the preliminary report of the excavator, Tolna-Mözs TO03 and its surroundings was the largest among the currently known Balaton-Lasinja settlements in Transdanubia, with only a couple of graves of the culture. The archaeological remains have not been evaluated and published yet.

Studied grave:

Feature 306/ STR 369 (grave 190, TOLM_2)

This burial (without grave goods) belongs to the Balaton-Lasinja culture, according to the observations of the excavator T. Horváth.

She was an adult individual (23-30 years old) with indifferent osteological sex determination, but genetically proved to be a female. She was found under the floor of a Balaton-Lasinja house, lying on her back in stretched position.

The anthropological evaluation of the human remains was carried out by Kitti Köhler (unpublished).

Törökszentmiklós-Tiszapüspöki, Karancs-Háromág, site 3.

The multi-period archaeological site of Karancs-Háromág was excavated in 1999-2000 by the archaeologists of the Damjanich János Museum (Szolnok). The rescue excavations brought to light 175 archaeological features on 3.7 hectares of research area. The earliest occupation of the site could be assigned to the Early Neolithic Körös culture, which 68 pit and 16 burials belonged to [57, 58].

Studied graves:

Feature 83/STR 137 (TOSM 1)

Grave of a ca. 20-22 years old female individual.

Feature 165/STR 315 (TOSM_3)

Grave of a ca. 11 years old child, genetically male.

Törökszentmiklós, road 4, site 3.

The Neolithic-Copper Age site was excavated by Tibor Paluch (Móra Ferenc Museum, Szeged).

Studied graves:

Feature 147 (GEN_68)

Genetically female individual.

The skeleton was radiocarbon dated in the frame of this project to the Early Neolithic Körös period: 5706-5541 cal BCE with 94.5% CI (6700±40 BP, Poz-83628).

Grave 191 (GEN_67)

Genetically male individual.

The skeleton was radiocarbon dated in the frame of this project to the Early Copper Age (Tiszapolgár) period: 4444-4257 cal BCE with 94.5% CI (5480±35 BP, Poz-83629).

Versend-Gilencsa

The site Versend-Gilencsa was excavated in 2006-2007 by V. Voicsek from the Janus Pannonius Museum (Pécs). Traces of 21 Neolithic building structures and 26 settlement burials came to light on the investigated area. The pottery style of the Neolithic households showed a mixed characteristics of the early Vinča, early LBK and early Sopot cultures. 25 graves were involved into the ancient DNA analyses out of the excavated 27 graves in the German-Hungarian DFG project (AL 287-10-1). We had four skeletons radiocarbon dated in the CEZA laboratory of Mannheim, their two-sigma values fall between 5300-5150 cal BCE.

Studied graves:

Feature 415 (VEGI_1)

Grave of an adult female individual (both osteologically and genetically).

The skeleton has the 95.4% CI radiocarbon date of 5369-5224 cal BCE (6321±28 BP, MAMS-14830).

Feature 1039 (VEGI 3)

Grave of an adult male individual (both osteologically and genetically).

Feature 1561 (VEGI_17)

Grave of an adult female individual (both osteologically and genetically).

The basic anthropological evaluation of the human remains was carried out by Zsuzsanna K. Zoffmann (unpublished).

Veszprém, Jutasi út

In the crossroad of the Jutasi and Munkácsy M. street, 15 graves were unearthed from the Neolithic and Copper Age in 2003 by J. Regenye (Laczkó Dezső Museum). Eight graves belong to the Neolithic Lengyel culture and four to the Middle Copper Age Balaton-Lasinja culture. The members of the small grave group were richly furnished with grave goods (especially noteworthy is grave 5). Graves 1-2 and 6-7 were double burials. The settlement was densely inhabited, but the area of the graves remained undisturbed. Seven out of eight Lengyel skeletons were found in crouched position, facing toward south, and one skeleton (grave 8) laid on her back. From the four Balaton-Lasinja graves, one double grave (numbers 13-14) was covered with calcar stones, which shows a unique rite in Transdanubia [59].

Studied graves:

Grave 2 (feature 98, VEJ_2)

Grave of a maturus-senilis male individual (50-70 years old) [60]. The genetic results confirm the osteological sex determination. The burial was dated to the Late Neolithic Lengyel period, based on the grave inventory (vessels and stone tools).

Grave 4 (feature 71, VEJ_4)

Grave of a juvenis (17-19 years old) individual with indifferent sex morphological markers [60], and male genetic results. He was found in right-crouched position, with one Lengyel type vessel in the grave.

The skeleton was dated to 4796-4685 cal BCE with 95.4% CI (5861±26 BP, MAMS-14827) [36], and so the dating of the burial to the Late Neolithic Lengyel period was confirmed.

Grave 5 (feature 219, VEJ_5)

Grave of a maturus male individual (50-60 years old) [60]. The osteological sex determination could be confirmed by the genetic analyses. The burial was assigned to the Late Neolithic Lengyel culture, based on rich inventory of grave goods.

Grave 9 (feature 280, VEJ_9)

Grave of an 8-9 years old child [60], genetically male, found in right-crouched position.

The skeleton was radiocarbon dated: 4339-4237 cal BCE with 95.4% CI (5418±29 BP, MAMS-14828) [36]. The burial was assigned to the Balaton-Lasinja period based on the grave goods (pottery, shell beads, stone tools) and the radiocarbon chronology.

Grave 15 (feature 562, VEJ_12)

Grave of a maturus (50-60 years old) male individual [60], found in left-crouched position. The genetic results confirm the osteological sex determination. The burial was assigned to the Late Neolithic Lengyel culture, based on stratigraphically observations.

Vésztő-Mágor

The excavation of the tell settlement of the late Szakálhát and the Tisza culture and the cemetery of the Tiszapolgár culture took place in 1972-1976. Leader of the excavation was Katalin Hegedűs [61, 62, 63].

Studied grave:

Grave 32 (inventory nr. 8413, VSM_3)

Grave of a maturus, osteologically male individual, who is genetically also male. The body was found in crouched position, lying on right side in E-W orientation, without any grave good.

2. Newly included Chalcolithic sites in Hungary

Abony, Turjányos-dűlő (Pest county)

The site Abony, Turjányos-dűlő (archaeological site Nr. 36) is located in central Hungary, about 15 km west of the Tisza River. On more than 136.000 m² surface circa 450 features were excavated between 2004 and 2008 by the Archaeological Institute of HAS. Leaders of the excavation were: Tibor Marton in 2004, Balázs Hansel in 2005, Gábor Serlegi, Szilvia Fábián in 2006-2008.

The excavated features are dated mostly to one archaeological period, the so-called Protoboleráz horizon, immediately preceding the Baden cultural complex at the beginning of the Late Copper Age. Beside the Protoboleráz period, one grave of Middle Copper Age Bodrogkeresztúr culture, a few settlement features from the Sarmatian period appeared at the site [64-69].

The majority of the settlement features were different kind of pits (storage pits and pit-complexes, postholes, hearths) indicated that the western part was the domestic area of the settlement. Among the groups of features of the domestic occupation, empty areas could be identified that were surrounded primarily by storage pits and regular pits. In many cases, these pits also contained a great amount of daub rubble and in situ collapsed vessels. The location and relationship of the pits, which contained many pieces of daub with imprints of wooden stick and boards may suggest that these relics have belonged to the structure of former houses or dwellings.

In the eastern part of the site, mainly elongated pits with sharp V section were found which could have served some function other than storage or clay extraction. We assume that these pits might have been necessary features for a special work process. This might have been the so-called 'economic' area of the settlement.

Beside the 'domestic' and 'economic' units of the Copper Age village, the 'sacral district' of the settlement was unearthed as well, comprising two groups of nine pits with special depositions/function. The two groups of features are different in all aspects from the rest of the site. The layers of these deep sacrificial pits contained pottery depositions, animal skeletons and eight of them have comprised special depositions of human remains of 48 individuals. The assemblage is even more special due to the presence of pits that include in clearly separable layers regularly buried and "thrown-in" human skeletons, partial skeletons, skulls, traces of fire, animal bones, intact vessels and sherds.

Studied features:

Feature 250.

It was a regular, round, beehive-shaped pit. Two human skeletons, an adult man (S-5) and a child (S-6), were found at a depth of 0.90–1.00 m below surface level. The cross-section shows that the

skeletons lie at the level of a yellow clay layer. Animal bones were lying above them and cattle horns were found directly below the two skeletons. At the bottom was a hole of 30 cm in diameter in the middle of the pit, containing a cattle skull and below, ceramic fragments with remains of an infant.

Feature 250/S6 (GEN_60)

Skeletal reimans of a 45-55 years old, genetically and osteologically male individual [69].

Feature 263.

It was a regular, round, beehive-shaped pit. The fill contained alternating layers of black humus with charcoal and yellow "sterile" clay of varying thickness. It is the smallest feature of the northern group of pits. However, this pit contained the largest number human remains of at least 23 individuals – (10 females, 7 males, 6 subadults). A top layer contained parts of human skeletons, incomplete and stumped skeletons, burnt animal bones and sherds. Underneath crouched skeletons of adults, children and neonates were deposited in more layers surrounded by animal bones, intact and vessels, fragments of pottery, rubbing and grinding stone and shells. At the bottom partial skeletal remains and horns of cattle and caprinae (bovids) placed in a pile and under them a posthole-like hollow was dug at the bottom of the pit.

Feature 263/S25 (GEN_61)

Skeletal reimans of a 35-45 years old, genetically and osteologically male individual.

Feature 263/S30 (GEN 62)

Skeletal reimans of a 45-55 years old, genetically female individual.

Feature 263/S34 (GEN_63)

Skeletal reimans of a 45-55 years old, genetically and osteologically male individual [69].

Alsónémedi (Pest county)

1250m² large, completely excavated cemetery of the Baden culture with 40 inhumation graves. Some of the graves were double burials, in one of them two individuals were buried with two cattle. Rich grave goods were discovered in part of the graves (pottery, copper pearls, copper puncher, shells, stone tools). The excavation was led by József Korek (Hungarian National Museum) in 1949. Besides the Copper Age graves, 19 Sarmatian and 4 Early Bronze Age cremation graves were unearthed [70].

Studied graves:

Grave 24. (GEN_16a)

Crouched burial of a 30-35 years old, genetically female individual.

Grave 53. (GEN_17a)

Crouched burial of a ca. 30 years old male individual. Grave goods: 5 Dentalium (shell) beads on the neck. (KOREK 1951, Abb. XIII. 8). This grave was radiocarbon dated in this project and the cultural assignment to the Baden period was supported by the result (3360-3100 cal BCE with 95,4% CI (4520±35 BP, Poz--83635).

Balatonlelle-Felső-Gamász (Somogy county)

A small graveyard of the Baden culture was excavated in 2002 at Balatonlelle that contained 23 graves, with very rich graves among them. The graves were disturbed by Middle Age objects and the deep ploughing. 200-250 meters away from the cemetery a Baden period settlement was excavated as well (site name: Balatonlelle-Országúti dűlő; M7/S-16). Excavator was Sófalvi András (Rippl Rónai Museum, Kaposvár). Other periods on the site: Neolithic, Migration period, Árpád Age settlement, Early Copper Age and 7th century AD graves [71,72].

Studied graves:

Grave 5. (STR 507, GEN 21)

Disturbed grave of a 40-59 years old male individual, found in crouched positions. Grave good: 1 decorated bowl, 1 jug,

Grave 9. (STR 536, GEN 22)

47-51 years old male individual in crouched position. Grave goods: 2 cups, 1 flint arrowhead, 1 sherd, 1 part of a bipartite bowl.

Grave 17. (STR 407, GEN23)

50-54 years old male individual in crouched position. Grave goods: 4 different blades, 8 flint arrowheads, 13 vessels in a semi-circle in the southern part of the pit. These were one bipartite bowl, one small jug, one pot, one bowl, one handled bowl, one larger jug and 7 mugs underneath. This grave was radiocarbon dated in this project, resulting in a Baden period date (3335-3025 calBCE with 95,4% CI (4465±30 BP, Poz-83637).

Grave 23. (STR 478, GEN_24)

40-49 years old male individual in crouched position. Grave goods: copper scraps, one goblet, one stone axe, two blades, one cattle tooth, one copper awl, one grindstone. 12 vessels were deposited in three groups little farther from the skeleton.

Budakalász-Luppa csárda (Pest county)

Budakalász was an emblematic site of the Baden culture, with an exceptional large number of graves (436). It was a multiritual cemetery (inhumations, cremations, double and triple burials, symbolic and empty graves, mixed rite grave). Rich grave goods were excavated in many of the graves (pottery, wagon models, copper objects, stone and shell beads, stone tools). The cemetery was most probably used for ca. 150-200 years long by the elite of the Baden culture. The whole area of the 9000 m² large cemetery was excavated by Sándor Soproni (Ferenczy Museum, 1952-61). Besides the Copper Age graves, one Early Bronze Age and two Early Iron Age graves were unearthed [73, 74, 75].

Studied graves:

Grave 33. (GEN_12a)

Flexed burial of a 40-50 years old male individual. Grave goods: 1 decorated pitcher, 1 undecorated goblet, fragment of a pot, 3 shells.

Grave 124. (GEN_13a)

Flexed burial of a 40-x years old male individual. Grave good: one spoon with handle (ladle).

Grave 203. (GEN_14a)

Contracted burial of a 48-56 years old male individual. Grave goods: one decorated goblet, 1 decorated jug.

Grave 319. (GEN_15)

Flexed burial of a 40-50 years old male individual. Grave good: one ladle, one copper chisel. this grave was radiocarbon dated in this project (3365-3105 calBCE with 95,4% CI (4545±35 BP, Poz-83634).

Nemesnádudvar-Papföld (Bács-Kiskun county)

543 features were excavated in 2009-2010 from the Baden, Celtic, Sarmatian and Árpád periods at this site, situated along the trace of the M9 highway. Complete cattle skeletons were discovered in some of the features of a former Baden age settlement, and in another pit skeletal remains of small ruminants were unearthed. Excavator György V. Székely found among the Copper Age settlement features two Copper Age burials in contracted positions [76].

Feature 173. (Grave 2/ SNR 0230, GEN_49)

Adult (genetically) male individual in contracted position. Grave goods: 1 blade, 1 decorated two-handled jar. The specialty of the jar is that it is a "depas amphikypellon" type jar but it has Baden-type cannelure decoration on its middle part. The skeleton was dated in this project, and placed the grave to the Hunyadihalom period, preceding the Protoboleráz and Baden periods (4230-3965 calBCE with 95,4% CI (5230±40 BP, Poz--83638).

Vámosgyörk (Heves county)

The excavation of the site was led by Csilla Farkas (Dobó István Museum) in 1997. Copper Age graves were clustered in three different areas of the site: in settlement pits of the Baden culture, a second grave group with disturbed burials, and a separate graveyard of 12 graves with animal burials. Further discovered periods on the site are Early Bronze Age, Scythian period and Árpád Age. [77, 78]

Studied grave:

Grave 36/2. (GEN_55)

Grave with a maturus female age skeleton in contracted position. An Árpád Age child grave disturbed the Copper Age burial. No grave goods were found beside the skeleton, stray sherd and stone were found in the filling of the grave.

Vörs (Somogy county)

Three graves were found at the site in 1952. Two of them belonged to the Copper Age, one to the Celtic period. One of the Copper Age burials was excavated by archaeologist Tamás Pekáry from the Balaton Museum [79, 80, 81].

Studied grave:

Grave 2. (Vors_1)

Female skeleton in contracted position with grave goods: copper diadem, shell necklace, 2 vessels.

3. Germany

Erwitte-Schmerlecke, Germany, Late Neolithic

I1560/SCHM2 (grave II, 3500-2900 BCE)

The Late Neolithic site of Erwitte-Schmerlecke is located in the Soester Boerde, Kreis Soest, Westphalia, Germany. The Soester Boerde is part of the Hellweg region, a loess zone already populated during LBK times. At Erwitte-Schmerlecke, two collective graves were excavated between 2009 and 2013, funded by the German Research Foundation [82, 83, 84]. Furthermore, probably End Neolithic and Bronze Age single grave burials with circular ditches were found in immediate surroundings. The collective graves of Erwitte-Schmerlecke belong to the type of the so called gallery graves of the Wartberg Culture [85]. They measured ca. 21 and 25 m in length and 2 to 5 m in width and were sunken into the ground; therefore only barely visible at their time of use. This and the fact that they were built of large limestone slabs made the preservation conditions for bones excellent. Anthropological and palaeopathological analysis is still in progress [86, 87, 88]. Sixty-six samples (46 from grave II, 20 from grave I) were AMS dated, most deriving from human

bones, but also eight animal bones (tooth pendants from carnivores and herbivores), and three charcoal pieces. Very few results show an eventual beginning of burying around 3600 BCE (keeping in mind possible reservoir effects, secondary burials and/or contamination), but most data belong to the expected time span of use between 3500-2900 BCE. One of two samples produced genome results.

4. Iberia

El Prado (Pancorbo, Burgos)

El Prado de Pancorbo [89] is an open-air Early Neolithic site located at the bottom of a valley next to a natural corridor in the Sistema Ibérico mountain range that connects the Duero and Ebro biogeographic regions. The complete settlement, that had an extension covering 0.30 hectares, was excavated in two seasons, in 2013 and 2014. A total of 51 features were uncovered, including 11 storage pits, 35 pits, 2 graves, 2 watering hole and 1 furnace. The spatial organization in two zones, with empty, intermediate areas, suggests the existence of two different occupational units. The homogeneity of the radiocarbon dates obtained points to a short period of human activity around 5300-4690 cal BCE. There are three AMS dates, one from a charcoal sample (E-40) from the "Polynesian furnace" (6,220±30 BP, 5,295-5,065 cal BCE), one from a human bone from grave E-06 (5,880±30 BP, 4,820-4,690 cal BCE) and other from a human bone from grave E-14 (6,170±30 BP, 5220-5030 cal BCE).

E-06-Ind-1 and E-14-Ind2

The two graves show a similar design, with an oval-shaped pit with several excavated containers and a two-meter diameter. One adult woman was buried in each grave; one was estimated to be 40-45 years-old (E-06) and the other one to be 48-56 years-old (E-14). Both women have no direct relationship of kinship. Their bodies were manipulated before the final inhumation and arranged in a cramped position. The anthropological analysis of the E-14 individual reveals a violent blow on the frontal bone. In this grave, several implements, including hand mills, a polished stone tool, a flint from a harvester's sickle and animal bones were found. They suggest a symbolic relationship with the harvesting cycle and they could be remnants of a funeral feast. The palinological analysis reveals the existence of nearby cereal fields. The pottery findings associated to the site show affinities to that found at the Ebro Valley and the Mediterranean region.

Dolmen Del Alto De La Huesera (Álava)

This megalithic site is located in the county of the Alava Rioja (Basque country), in the municipality of Laguardia-Guardia, at the south of the historical territory of Alava. The site is 614 meter above sea level. It is a megalithic tomb formed by a chamber with seven vertical slabs and an eight-meters long corridor. The corridor has a lintel section and another one in the outside,

demarcated by small slabs, with the exception of a big slab on the Western side. It was built at the end of the Neolithic (around 5000 BP) and was continuously used until the Bronze Age (around 3550 BP).

The site was discovered in 1947 by Domingo Fernández Medrano and excavated by him in 1948. Between 2010 and 2014, new excavations were performed by José Antonio Mujika Alustiza and Javier Fernández Eraso [90].

The dolmen collapsed in ancient times; the stone cover fell down into the chamber, and this likely triggered the abandon and re-structuring of the burial monument; a lateral access was opened up to the linteled area of the corridor. In this area, an anthropomorphic stela was discovered, in which a hand holding a kind of halberd and two knives can be seen. From the inside the chamber and the corridor, skeletal remains of 130 individuals were retrieved, along with arrow points and sylex flints, as well as a bronze burin, a gold plaque, a bone point and some ornaments made in variscite, bone, lignite and limestone.

A total of 21 radiocarbon dates were generated from the human remains, and yielded dates between the Chalcolithic and the Bronze Age: 3550±30; 3850±30; 4010±30; 4050±30; 4050±30; 4080±30; 4100±30; 4230±30; 4290±30; 4300±30; 4320±30; 4320±30; 4320±30; 4340±30; 4350±30; 4390±30; 4410±30; 4430±30; 4450±30; 4450±30; 4450±30; 4520±30 BP.

Dolmen "El Sotillo" (Álava)

El Sotillo megalithic site is located in the Alava Rioja county (Basque country), between the limit of Laguardia-Guardia and Leza municipalities, at the south of the historical territory of Alava. The site is 617 meters above the sea level. It was discovered in 1955 by Domingo Fernández Medrano and excavated by himself, José Miguel Barandiran and Juan M. Apellániz in 1963 [91].

It is a megalithic tomb with a corridor and an almost circular chamber, formed by nine slab stones, a corridor and a tumulus of eleven meters of diameter. During the excavation, numerous lithic tools were uncovered, including six pedunculated arrowheads of silex, a bone and a metal arrowheads, a metal burin, retouched flakes, two fragments of foliaceous projectile points, etc. There are some Bell Beaker pottery remains and a cup with incised decorations.

The remains of thirteen individuals, including eleven adults (six of them males) were retrieved. The radiocarbon dates placed the initial use of the site at the Late Chalcolithic period, the Bell Beaker period (4390±30, 4350±30, 4040±30, 4000±40 BP). After a hiatus of about half a millennium, the usage of the structure as funerary place increased during the Middle Bronze Age period (3550±30, 3430±30, 3380±30, 3360±30, 3360±30, 3320±30, 3160±30, 3120±30 BP), with one date from the Late Bronze Age (2740+30 BP).

La Chabola De La Hechicera (Álava)

The dolmen of La Chabola de la Hechicera [91] is located in the Alava Rioja county, in the municipality of Elvillar. It is a corridor megalithic burial composed by a circular chamber formed by eight slab stones, and a corridor delimited by six slabs and covered by a large slab stone. It was erected during the Late Neolithic and was used in different periods, until the Bronze Age. It was discovered in 1935 by Álvaro de Gortazar and has been excavated in several campaigns by different researchers (1936 José Miguel Barandiaran, 1947 Carlos Sáenz de Tejada, Álvaro Gortazar y Domingo Fernández Medrano, 1974 Juan María Apellániz and 2010-2011 José Antonio Mujika y Javier Fernández Eraso).

During these works, the remains of at least 39 individuals have been retrieved. Sylex arrowheads, personal ornaments (such as necklace beads and pendants made from different materials), an idol made of bone and pottery remains (including a well-preserved Bell Beaker cup in the Ciempozuelos style) were also retrieved. Twelve different radiocarbon dates were generated, yielding dates from the Late Neolithic to the Bronze Age: 3170±130; 3280±40; 4380±40; 4420±30; 4430±40; 4440±40; 4450±40; 4650±40; 4670±40; 4940±30; 4980±30 BP.

Las Yurdinas II (Álava)

The Las Yurdinas II site is located in the North slopes of the Cantabrian range, south of the municipality of Peñacerrada in Alava. The site was excavated by Javier Fernández Eraso in 1999-2000. It is located 907 meters above sea level. It is an open rock shelter in the Cretaceous limestone, oriented to the West. It measures 20 meters (North-South) and has about 3.5-4 meters of depth. The bottom is solid rock without any filling; two meters from the base of the wall, at the North side, there are some dark-ochre paintings representing a female silhouette and a bovid head. At the North side of the rock shelter there is a crack (1.70 meters long by 1.40 meters wide) that leads to a small chamber that was used as a funerary deposit [92].

This chamber contained the remains of 95 individuals, including both males and females and all ages. Different radiocarbon dates on these remains place the use of the site to the Chalcolithic, between 4390± 80; 4360± 40 and 4290± 40 BP. The bodies were accompanied by burial goods, including arrowheads, silex flakes and burins, bone points. and a necklace made by lignite, limestone and muscovite mica. Remains from three ceramic vessels were also retrieved.

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Supplementary note 3: Y chromosomal data

Y chromosomal haplogroup assignment of 82 Neolithic and Chalcolithic samples from Hungary (~6000-2900 BC), Germany (~5500-3000 BC) and Spain (~5500-2200 BC).

Table S3.1: Summary of Y-chromosomal data of all newly reported or in this study revised European Neolithic and Chalcolithic male individuals.

Harvard ID	Collaborator ID	Population	Y haplogroup	Reference (if published)
I2374	TÖSM_3a	Körös_EN	G	
I4971	TIDO2a	Körös_EN	I2a2	
I1876	BAM4a	Starčevo_EN	G2a2a1 (x G2a2a1b)	
I1877	BAM13b	Starčevo_EN	G2a2b2b	
I1878	BAM17b	Starčevo_EN	H2	
I1880	LGCS_1a	Starčevo_EN	G2a2b2b1a (x G2a2b2b1a1)	
I2739	GEN_18	LBKT_MN	G2a2b2b1	
I1904	BAL_25b	LBKT_MN	G2a2a1	
I1882	BUD_4a	LBKT_MN	G2a2b2a	
I1883	TOLM_4a	LBKT_MN	G	
I2743	CEG03b	ALPc_MN	G2a2b2a	
I2744	CEG07b	ALPc_MN	G2a2b2a	
I2375	TISO_1b	ALPc_MN	I2a2a1b1	
I2376	TISO_11a	ALPc_MN	Н	
I2377	TISO_13a	ALPc_MN	I2a2a	
I2378	HELI_2a	ALPc_MN	I (x I1)	
I2379	HELI_11a	ALPc_MN	I2a2a1b (x I2a2a1b1)	
I2382	MEMO_24b	ALPc_MN	CT	
I2383	HAJE_1a	ALPc_MN	G2	
I2384	HAJE_7a	ALPc_MN	I2c	
I3535	HAJE_10a	ALPc_MN	I (x I1)	
I4187	EBVO_5a	ALPc_MN	CT(x G, I, J)	
I4188	POPI_5a	ALPc_MN	I2a2a	
I1887	VEGI_3a	Vinca_MN	H2	
I1889	SEKU_1a	Vinca_MN	G2a2a1a2a	
I1896	SEKU_10a	Vinca_MN	G2a2b2a1a	
I2746	VSM_3a	Tisza_LN	G2a	
I0449	Gorzsa18	Tisza_LN	I2a1	
I2387	KOKE_3a	Tisza_LN	I	

I1890	FAGA_1a	Sopot_LN	I (x I1, I2a2)	
I4185	ALE_4a	Sopot_LN	F(x I)	
I1893	ALE_14	Sopot_LN	G2a2b	
I4183	SZEH_5a	Sopot_LN	G?	
I1899	VEJ_2a	Lengyel_LN	C1a2	
I1900	VEJ_4a	Lengyel_LN	E1b1b1a1b1	
I1901	VEJ_5a	Lengyel_LN	G2a2a1	
I2352	VEJ_12a	Lengyel_LN	Н	
I1902	FEB_3a	Lengyel_LN	J2a	
I1903	BAL_3a	Lengyel_LN	H2	
I1906	CSAT_19a	Lengyel_LN	Н	
I1905	CSAT_25a	Lengyel_LN	I2c	
12793	GEN_67	Tiszapolgar_ECA	I2a2a1b (xI2a2a1b1)	
I2353	PULE1.10a	Tiszapolgar_ECA	I2a	
I2354	PULE1.13a	Tiszapolgar_ECA	G2a2b2a1a1c1a	
I2356	PULE1.22a	Tiszapolgar_ECA	G2a2b	
I2395	PULE1.9a	Tiszapolgar_ECA	G2a2b	
I2394	VEJ_9a	Lasinja_CA	C1a2	
I2783	GEN_49	Hunyadihalom_CA	C1a2a	
I2788	GEN_60	Protoboleraz_LCA	G2a2b2a	
I2789	GEN_61	Protoboleraz_LCA	I2c	
I2791	GEN_63	Protoboleraz_LCA	I2c	
I2366	GEN_12a	Baden_LCA	G2a2b2a1a1b1	
I2367	GEN_13a	Baden_LCA	G2a2b2a1a	
I2368	GEN_14a	Baden_LCA	G2a2b2a1a1c1a	
I2369	GEN_15a	Baden_LCA	G2a2b2a1a1c1a	
I2371	GEN_17a	Baden_LCA	G2a2a	
I2752	GEN_21	Baden_LCA	I2a1	
I2753	GEN_22	Baden_LCA	I2a1a1a	
I2754	GEN_23	Baden_LCA	I2	
I2755	GEN_24	Baden_LCA	I	
I2037	HAL39b	LBK_EN	G2a2a1	
I0048	HAL25	LBK_EN	G2a2a1	[1], this study
I0056	HAL14	LBK_EN	G2a2a	[1], this study
10659	HAL2	LBK_EN	G2a2a1 (x G2a2a1a)	[1], this study
I0821	HAL24	LBK_EN	G2a2a1	[1], this study
I0795	KAR6	LBK_EN	CT	[1,2], revised here
I0551	SALZ3B	Germany_MN	G2a2a1	[1,2], revised here
I0581	MIR5, MIR6	Iberia_CA	I2a2a	[2], revised here

I5838	MIR202-037-n105	Iberia_CA	I2a2a
I2014	HAL15a	LBK_EN	G2
I2020	HAL20b	LBK_EN	G2a2a
I2021	HAL21a	LBK_EN	G2a2a?
I2026	HAL27a	LBK_EN	G2a2a
10802	SALZ77A	Germany_MN	IJK (x J)
I1594	Bla28	Blatterhohle_MN	R1b1a
I1593	Bla16	Blatterhohle_MN	R1b1a
I1565	Bla8	Blatterhohle_MN	I2a1
I2467	Inventario 0/4	Iberia_CA	I2a2a
I2473	ES-6G-110	Iberia_CA	I2a2a
I1975	5K18	Iberia_CA	I2a2
I1976	ES.1/4	Iberia_CA	I2
I3269	LY.II.A.10.15066	Iberia_CA	I2a2a2a
I3272	1K11	Iberia_CA	I2a2
I3276	LHUE2011.11	Iberia_CA	G2a2a

Y-chromosomal SNP calls were compared to the ISOGG Y-tree (www.isogg.org) version 12.34, updated on 5th February 2017.

Here we present the detailed Y-chromosomal profiles of 63 Neolithic-Chalcolithic male individuals from present-day Hungary, including four previously published results [1, 3]. The most dominant haplogroup is G (G2a) (ca. 40%) throughout the studied period of ~6000-2850 BC, as it was presumed by PCR based Y-chromosome analyses [4]. The diversity of the G2a chromosomes cannot be judged entirely, due to different levels of DNA preservation and uneven coverage of the captured SNPs per sample. However, some level of variability is seen in the Early and Middle Neolithic (EN and MN) Carpathian Basin already (G2a2b and G2a2a subgroups), similarly to the Y-chromosomal variability of western Anatolia [2]. The second most frequent haplogroup was haplogroup I (I2) in the Hungarian transect (ca. 32%), which also shows some variability (I2a1, I2a2, I2c). The I2a haplogroups might originate from the local hunter-gatherer population, since they were previously described in individuals with hunter-gatherer genomic profiles [1, 3]; on the other hand, I2c could also come from the Near East, as it has been detected in the 6500-6200 BC period of western Anatolia [2]. Samples previously reported as F* [4, 5], could mostly be further categorized into subhaplogroups H2 (BAM17b, VEGI3, BAL3, CSAT19). Aside from these major haplogroups, C, E and J2 were detected in the Hungarian transect, all the three showing rather sporadic prevalence. In general, the Hungarian Neolithic-Chalcolithic Y-chromosomal dataset shows close connections to the ancient Anatolian data [2], and has virtually no affinity to the known Natufian and PPNB Y-chromosome pool of Levant [6]. It is noteworthy that the nowadays

most frequent European haplogroups R1a and R1b have not been detected in Hungary before the Early Bronze Age [5].

In the EN-MN Neolithic transect from today's Germany, haplogroup G (G2a2a) is the most frequent (60.8%), having probably spread from the Near East to Germany via the Carpathian Basin. Furthermore, the sporadic appearances of Y haplogroups R, T1 and I (I2a) are detected in the relatively small EN-MN Y-chromosomal dataset (n=23) [1, 2].

Similarly to the local mtDNA gene pool [7], the Iberian Neolithic-Chalcolithic Y-chromosomal dataset (n=20) shows significant differences from Central Europe as well. This are seen on the predominance of haplogroup I (mostly I2a) in 71% and the low level of haplogroup G2a (14%), in addition to the sporadic occurrence of H2 and R1b (the latter already existing in the EN) [1, 2].

Detailed Y chromosomal haplotype descriptions

Samples from Hungary:

I2374/TÖSM3a: G

This Körös_EN individual was derived for three SNPs (M3264, CTS2136, M3628) characteristic for haplogroup G and for one downstream position Z3220, defining G2a2b2a1. The ancestral positions on the G branch do not contradict to the subhaplogroup definition G2a2b2a1, but the lack of derived supportive SNP calls within the G clade does not allow a secure subhaplogroup definition. Thus he could be designated as G.

I4971/TIDO2a: I2a2

This Körös_EN individual was derived for 20 SNPs (CTS88, PF3640, L758, F3665, L1197, CTS4209, CTS4848, PF3742, CTS5946, CTS6265, PF3778, L751, CTS9618, CTS9860, CTS10058, PF3796, PF3814, Z16987, PF3828, PF3829) characteristic for haplogroup I. P218 and L368 defined I2a2. Positions P222 and M223 showed ancestral alleles for I2a2a. Thus he could be designated as I2a2.

I1876/BAM4a: G2a2a1(x G2a2a1b)

This Starcevo_EN individual was derived for SNPs PF3182 (G2a2a) and PF3170 (G2a2a1), as well as nine upstream SNPs defining G2 (Z3100, CTS4413, M3531, PF2971, CTS4703, M3533, PF2976, M3579, Z6103) and six SNPs defining G2a (F4086, M3251, PF2863, CTS6026, M3331, PF2991). He was ancestral for G2a2a1b (L91, PF3246, S285), and was not further typed within the G2a2a1 subgroup. Thus, based on these analyses, he could be designated as G2a2a1 (x G2a2a1b).

<u>I1877/BAM13b: G2a2b2b</u>

This Starcevo_EN individual showed the derived allele for 33 SNPs in the G2 cluster (also P287) and 13 SNPs defining the upstream G2a subgroup (also P15). Furthermore, he was derived at the positions PF3321, F1175, F1429, PF3392, PF3400, PF3418, all of them defining G2a2b2b. This individual was ancestral for downstream SNPs of G2a2b2b (F935, F1671, F1932, PF3394), and PF3401 defining G2a2b2b1a, and for SNPs downstream of this subgroup. Thus, he could be designated as G2a2b2b.

<u>I1878/BAM17b: H2</u>

This Starcevo_EN individual was derived eight SNPs defining H (M2713, Z13964, M2896, M2942, M2945, M2955, M2992, M3070), and for SNPs P96, L285, L286 defining subhaplogroup H2. Congruently, he was ancestral to several H1 and H3 defining SNPs. Subgroups H2 were not typed in our analyses. Thus, he could be designated as haplogroup H2.

<u>I1880/LGCS1a: G2a2b2b1a (x G2a2b2b1a1)</u>

This Starcevo_EN individual was derived for four SNPs (F1932, PF3394, PF3401, F2537) defining haplogroup G2a2b2b1a, and also derived for three SNPs (FGC7260, PF3359, F1429) defining upstream G2a2b2b. This individual was ancestral to PF3379, one of the representing SNPs of downstream subgroup G2a2b2b1a1. Thus, he could be designated as G2a2b2b1a (x G2a2b2b1a1).

<u>I2739/GEN18</u>: G2a2b2b1

This well preserved LBKT_MN sample could be detected at 732 SNPs with derived alleles on the Y-chromosome. He was derived for more than 80 SNPs defining haplogroup G, for 32 SNPs defining G2. He was also derived for nine G2a representing SNPs (F4086, L149.1, CTS1879, CTS6753, F2529, F3088, M3393, CTS11463, P15), and for G2a2 representing L1259. Subhaplogroup G2a2b2b was represented by derived alleles of ten SNPs (F795, PF3359, F1175, F1429, F1581, F1760, F2419, PF3413, PF3418, F1705). Furthermore, downstream terminal SNP F1193 defined subhaplogroup G2a2b2b1.

I1904/BAL25b: G2a2a1

This LBKT_MN individual was derived for more than hundred SNPs characteristic for G, 33 SNPs characteristic for G2, and for 11 SNPs characteristic for G2a. He was derived for eight G2a2a defining positions (PF3147, PF3151, PF3159, PF3165, PF3166, PF3181, PF3184, PF3185), and for G2a2a1 characteristic SNPs S11769, PF3155, S15710, PF3170. He was ancestral to SNP L91 defining G2a2a1b and also to M286 defining G2a2a1a. Thus, he could be designated as G2a2a1.

I1882/BUD4a: G2a2b2a

This LBKT_MN individual was derived for SNPs CTS946, CTS4454 defining G2a2b2a. Other, for G2a2b2a representative SNPs (like P303) were tested, but could not be detected. He was also

derived for upstream PF3141 (G2a) and further 19 SNPs characteristic for haplogroup G. Thus, he could be designated as G2a2b2a.

<u>I1883/TOLM4a: G</u>

This LBKT_MN individual was derived for SNPs defining G (P166, P136, Z3218, L521, Z3262, M3514). Thus, we can assign this individual to subhaplogroup G.

I2743/CEG03b: G2a2b2a

This ALPc_Szakalhat_MN individuals was derived for 24 SNPs characteristic for haplogroup G, 12 SNPs characteristic for G2 (PF2807, PF2835, M3446, F1393, PF2909, Z3274, CTS1868, CTS1900, CTS4264, CTS5666, CTS6316, F3220). Furthermore, three derived SNPs represented the G2a subhaplogroup (M3320, M3393, M3408) and three SNPs represented the G2a2b2a subhaplogroup (PF3332, PF3342, Page98). Thus, he could be designated as G2a2b2a.

I2744/CEG07b: G2a2b2a

This well preserved ALPc_Szakalhat_MN sample was derived for 31 SNPs characteristic for haplogroup G, five SNPs characteristic for G2 (PF2787, M3446, CTS7662, CTS10089, M3579). Downstream SNP U5 defined subhaplogroup G2a. Furthermore, derived alleles of six SNPs (CTS688, PF3329, PF3330, PF3342, CTS4454, Z3243) defined subhaplogroup G2a2b2a. Thus, he could be designated as G2a2b2a.

<u>I2375/TISO1b: I2a2a1b1</u>

This ALPc_MN individual was derived for eight SNPs characteristic for haplogroup I (L578, PF3661, FGC2415, CTS7831, L751, PF3797, L503, PF3837). He was also derived for L181, defining I2a2 and P221 defining I2a2a, and L702 defining I2a2a1b1. Thus, he could be designated as I2a2a1b1.

I2376/TISO11a: H

This ALPc_MN individual was derived for four SNPs defining haplogroup F (P134, P145, P160, P135), and for four SNPs defining haplogroup H (M2713, M2992, M3052, M3070) and one SNP representing the HIJK branch (F929). He was ancestral for one H defining SNP (M2826) and also for 22 downstream SNPs defining H3. Two downstream SNPs showed derived allele for subhaplogroups H1a2b1a (Z14350) and H3b (Z13904), but these were not supported by other ancestral alleles defining the same branches. Thus, he could be designated as H.

I2377/TISO13a: I2a2a

This ALPc_MN individual was derived for 46 SNPs representing haplogroup I. Furthermore, he was derived for SNPs P216, L37, L35, and L368 defining haplogroup I2a2, and for downstream SNP P220 characteristic for I2a2a. Further downstream derived positions (CTS10100 and L1228)

gave contradictory results. Therefore, further typing of this individual was not possible. Thus, he could be designated as I2a2a.

<u>I2378/HELI2a: I (x I1)</u>

This ALPc_MN individual was derived for four SNPs defining haplogroup I (L578, FI3, Z16985, PF3809). He was ancestral for L840 defining I1 and L417 defining I2b. He was derived in L1228 SNP defining I2a2a2a, but further positions do not support this suphaplogroup assignment. Thus, he could be designated as I (xI1).

<u>I2379/HELI11a</u>: <u>I2a2a1b</u> (x <u>I2a2a1b1</u>)

This ALPc_MN individual was derived for 33 SNPs defining haplogroup I. He was also derived for L460 defining I2a, and for P217, P218, L37, L181, L35 and L368, all representing I2a2. Downstream positions of P220, P221, P223, L36, and M223 define subhaplogroup I2a2a, and CTS10057, CTS10100 are the only two positions characteristic for I2a2a1b. He was ancestral for L702 that would define I2a2a1b1. Thus, he could be designated as I2a2a1b (x I2a2a1b1).

I2382/MEMO24b: CT

Sequencing of this ALPc_MN individual resulted only in 187 SNP calls on the Y chromosome. From the derived alleles, five defined macro group BT and six belonged to cluster CT. Based on the detected ancestral loci, haplogroups D, E, G, I, J, K could be excluded. Thus, he could be designated as CT (x D, E, G, I, J, K).

I2383/HAJE1a: G2

This ALPc_MN individual could be analyzed for 216 SNPs on the Y chromosome. He was derived for six haplogroup G defining SNPs (M3450, M3480, M3517, M3585, PF2793, PF3080) and for one representing SNPs (PF2929) of haplogroup G2. Thus, he could be designated as G2.

I2384/HAJE7a: I2c

This ALPc_MN individual was derived for 36 SNPs defining haplogroup I. SNP L68 defines downstream subhaplogroup I2. For the subgroup I1 characteristic SNPs (L64, M253, S63, L75, L840) showed ancestral alleles. Derived alleles of SNPs L596, L597, PF3893, S6631 defined subgroup I2c.Therefore, this individual could be designated as I2c.

<u>I3535/HAJE10a: I (x I1)</u>

This ALPc_MN individual was derived for 10 SNPs defining haplogroup I (CTS48, CTS674, PF3665, PF3687, CTS3517, CTS10058, PF3800, PF3817, PF3837, CTS10941) and L672, characteristic for I2a1a1a. Since he was also ancestral for L158 (I2a1a1), and two SNPs characteristic for I1 (S63, S65), therefore he could only be assigned to haplogroup I (xI1).

<u>I4187/EBVO5a: CT (x G, I, J)</u>

This ALPc_MN individual obtained call for derived alleles of four SNPs, characteristic for cluster CT (M5589, M5599, M5632, M5652, Y1526). No further downstream derived SNPs could be called. Downstream ancestral alleles were detected among representing SNPs of haplogroup G, I, and J. Thus, this individual could be designated as CT (x G, I, J).

I4188/POPI5a: I2a2a

This ALPc_MN individual was derived for 13 SNPs characteristic for haplogroup I (CTS646, CTS674, PF3641, FGC2413, L758, CTS4088, CTS7540, CTS8333, CTS9860, L503, PF3803, FGC7049, CTS11979), for P217, representing subhaplogroup I2a2, and P222 representing I2a2a. Thus, this individual could be designated as I2a2a.

<u>I1887/VEGI3a: H2</u>

This Vinca_MN individual was derived for SNP M2955 defining haplogroup H, and downstream SNPs P96 and M282, both defining H2. However, he was derived also for Z14345, representing H1a2b1a. He was ancestral for several other characterisitc SNPs of H1, H3, and also ancestral for 16 other SNPs defining subgroup H1a2b1a. Thus, he could be designated as H2.

I1889/SEKU1a: G2a2a1a2a

This Vinca_MN individual was derived for 26 SNPs defining haplogroup G, nine SNPs characteristic for haplogroup G2 (Z3100, F1239, F1393, M3491, PF2909, CTS9885, F3198, P287, Z6474), for four SNPs (F4086, Z3240, L31, P15) defining G2a and for the downstream SNPs S15710, PF3148 defining G2a2a1 and SNP PF3237 defining G2a2a1a2a. Thus, he could be designated as G2a2a1a2a.

I1896/SEKU10: G2a2b2a1a

This Vinca_MN individual was derived for the SNP PF3346, defining haplogroup G2a2b2a1a, and also for the upstream positions CTS946 and PF3346 defining G2a2b2a, and M3331 defining G2a, and for further four SNPs (PF2912, Z3274, M3573, M3626) representing subhaplogroup G2. He was ancestral to downstream position L497 defining haplogroup G2a2b2a1b. Thus, he could be designated as G2a2b2a1a.

I2746/VSM3a: G2a

This Tisza_LN individual was derived for eight SNPs (CTS1029, M3487, F1383, Z3262, CTS2517, CTS8531, CTS10723, M3628) characteristic for haplogroup G, and three SNPs (F1239, CTS1900, F3536) characteristic for G2. Derived SNP M3334 assigned this individual to the subhaplogroup G2a.

I0449/Gorzsa18: I2a1

This Tisza_LN individual was derived for 73 SNPs characteristic for haplogroup I. Two SNPs representing haplogroup I2 (M438, L68), L460 defining I2a, P37.2 defining I2a1. Within the subcluster I2a1 only SNP L1286 (I2a1a2a) showed derived allele, but CTS616 (I2a2a1) and L1228 (I2a2a2a) gave derived allele calls as well. Upstream SNPs in the I2a2 and I2a2a subhaplogroups disclaim the credibility of these latter two data. For the safe definition of I2a1a2a two more SNPs (CTS595, S21825) should have been detected. Thus, he could be assigned to subahplogroup I2a1.

I2387/KÖKE3a: I

This Tisza_LN individual had only 30 derived SNP calls. Three of them represent haplogroup I (P212, PF3766, PF3776), which haplogroup definition does not contradict to the call of 318 ancestral SNPs. S66 representing I1 showed ancestral allele. Thus, this individual could be designated as I (xI1).

<u>I1890/FAGA1: I (x I1, I2a2)</u>

This Sopot_LN individual was derived for five SNPs (PF3661, PF3666, PF3750, PF3815) defining haplogroup I. He was also derived for the downstream position L1228 belonging to subhaplogroup I2a2a2a. However, he was ancestral to I2a2 representative position P221, and also to I1 defining S107, S64. Therefore, this individual could only be assigned to haplogroup I (x I1, I2a2).

<u>I4185/ALE4a: F (x I)</u>

This Sopot_LN individual was derived for four SNPs representing cluster CT (M5595, M5611, M294, L977) and three SNPs characteristic for haplogroup F (P142, P146, M235). Further derived SNPs were detected in haplogroup G (M3555) and haplogroup J (CTS12047). However, ancestral allele calls were obtained for SNP M3567, also defining haplogroup G, and SNPs L758, PF3699, PF3726, PF3837 defining haplogroup I. Thus, he could be assigned to haplogroup F (x I).

I1893/ALE14: G2a2b

This Sopot_LN individual was derived for the SNPs M3307 and M3333, characteristic for subhaplogroup G2a and F2908 defining G2a2b. He was also derived for upstream SNP M3483 defining haplogroup G2, and M3489, PF3045, M3622 defining haplogroup G. This individual could be assigned to haplogroup G2a2b.

<u>I4183.L1/SZEH5a: G?</u>

This Sopot_LN individual was derived for two SNPs defining cluster BT (Z12005, M11779), and one SNP defining haplogroup G (M3588). No other derived alleles could be called.

I1899/VEJ2: C1a2

The following CT macro group defining SNPs were derived in this Lengyel_LN individual PF38, Y1462, PF143, M5588, PF228, CTS1181, M5597, M5612, Y1521, M5652, M5679, M5692,

M5705, M5713, M5714, M5723, CTS7922, CTS9948, M5767, M5775, M5782, Z17718, Z17720, L1492, M5809, PF1337. Downstream from CT, the SNP CTS3818 defined CF and the P184 defined haplogroup C. He was derived for CTS12440 representing G and Z6023 defining G2, however ancestral to other 31 G haplogroup defining SNPs. He was also ancestral for SNPs representing F (P142, P145, M235, P316, L132.1, P158), and M9 defining K. He was ancestral for SNPs of C subgroups like C1a1 (M8, CTS483), C1b1a1a (Z12438, K129, Z12443, K186, K187, Z12450, K417), and C2 (Z1453). He was derived for C1a2 SNP Y11464. Thus, he could be designated as C1a2.

I1900/VEJ4: E1b1b1a1b1

This Lengyel_LN individual showed derived alleles of seven SNPs (P172, M5422, Z15671, L504, M5533, PF1864, CTS11504) defining haplogroup E. Furthermore, he was derived for PF2115 defining E1b1b1a and position Z1061 defining E1b1b1a1b1. Further representative positions of this subgroup or its downstream SNPs failed to get captured and sequenced. Thus, this individual could be assigned to haplogroup E1b1b1a1b1.

I1901/VEJ5: G2a2a1

This Lengyel_LN individual was derived for 41 SNPs defining haplogroup G and further 16 SNPs defining G2. Subgroup G2a was defined by SNPs L149.1, Z3240, L31, F2274, F2529, F3088, M3397, U5. Subgroup G2a2a was represented by SNPs PF3147, PF3167, PF3175, PF3185 and G2a2a1 was defined by PF3155, PF3170. Thus, he could be designated as G2a2a1.

<u>I2352/VEJ12a: H (xH3)</u>

This Lengyel_LN individual was derived for SNP P145, defining haplogroup F, and SNP FGC2045, defining GHIJK cluster. He was also derived for SNPs M2713, M2896 characteristic for H. He was ancestral for nine SNPs defining H3 and for several subgroups of H1. Thus, he could be designated as H (xH3).

I1902/FEB3: J2a

This Lengyel_LN individual was derived for 11 positions defining J (PF4530, CTS3872, CTS5280, F2116, CTS7483, S19861, PF4575, CTS9877, CTS10446, PF4598, CTS10858) and for one SNP characteristic for J2a (L152). He was ancestral for J2b (M314), and failed to give calls at downstream positions of the J2a subgroup. Thus, he could be designated as J2a.

I1903/BAL3: H2

This Lengyel_LN individual was derived for seven haplogroup H characteristic SNPs (M2920, L901 M2955, M2992, M3010, M3052, M3070). He was also derived for the SNPs L279, L285, L3010 defining H2. Thus, he could be designated as H2.

I1906/CSAT19: H

This Lengyel_LN individual was derived for two SNPs characteristic for haplogroup F (P141, M235). He was also derived for SNP F929 defining cluster HIJK. He was also ancestral for the SNPs defining haplogroups J and I, but missing SNP calls failed to secure the basal K branch. Further five SNPs characteristic for haplogroup H (Z4205, M2896, L901, Z4309, M3070) and for one SNP Z13917 signalizing H3b could be detected on his Y chromosome. Contrasting to the SNP results found within the H branch, he was also ancestral for M2826, characteristic for H and 28 SNPs defining H3 and 15 defining H3b. Considering all the 2425 observed SNPs, he could be designated as H.

I1905/CSAT25:I2c

This Lengyel_LN individual was derived for 22 SNPs characteristic for haplogroup I (CTS88, FGC2416, PF3640, PF3641, FGC2413, L578, L756, PF3670, FGC2415, CTS1800, CTS2387, CTS4088, CTS5650, CTS7502, CTS7831, CTS8876, L751, PF3800, YSC0000272, Z16987, L847, CTS11540). He was also derived for SNP M438 characteristic for I2. He was ancestral to SNPs defining haplogroup I1 (L450, L81, L118). He carried the derived alleles for subgroup I2c (L596, M3934, PF3893, S6631). Thus, he could be designated as I2c.

<u>I2793/GEN67</u>: <u>I2a2a1b</u> (xI2a2a1b1)

This Tiszapolgar_ECA individual was derived for 62 SNPs characteristic for haplogroup I, L68 defining haplogroup I2, P126, L35, L37, L181 representing subhaplogroup I2a2. He also showed derived allele at CTS9183, defining I2a2a1. The detected derived SNPs at CTS10057 and CTS10100 are currently the only two positions defining I2a2a1b. He was ancestral to L702 (I2a2a1b1) and also to SNPs of I2a2b (L38, L39, L65.1). Thus, he could be assigned to I2a2a1b (xI2a2a1b1).

I2353/PULE1.10: I2a

This Tiszapolgar_ECA individual was derived for eight SNPs characteristic for haplogroup I (CTS48, L755, PF3660, PF3699, L772, PF3871, PF3775, PF3837) and one SNP (L460) characteristic for I2a. He was ancestral for I2b (L417) and I2c (L597) and also for I2a2 (P216). Thus, he could be designated as I2a.

<u>I2354/PULE1.13: G2a2b2a1a1c1a</u>

This Tiszapolgar_ECA individual was derived for 29 SNPs characteristic for G and 16 SNPs characteristic for G2. Seven derived SNPs (F4086, Z3240, L31, CTS6026, F2274, CTS6314, CTS9318) were characteristic for G2a. Derived allele of L190 signalizes subhaplogroup G2a2b, PF3325 represents G2a2b2. SNPs Z3243 and PF3342 are characteristic for subhaplogroup G2a2b2a. Furthermore, SNPs Z3220, PF3337 are representative for G2a2b2a1 and Z3423, CTS11388 for G2a2b2a1a1c1a. He was ancestral to several SNPs on the G2a2b branch, but none of these alleles were in contradiction with the haplogroup definition G2a2b2a1a1c1a.

<u>I2356/PULE1.22</u>: G2a2b

This Tiszapolgar_ECA individual was derived for 19 SNPs characteristic for G, six SNPs characteristic for subhaplogroup G2a (F744, PF2909, Z6023, CTS1868, L149.1, P15) and for L32 defining G2a2b. Thus, he could be designated as G2a2b.

I2395/PULE1.9a: G2a2b

This Tiszapolgar_ECA individual was derived for 14 SNPs defining haplogroup G, and for three SNPs (M3469, M3491, F3220) defining G2 subhaplogroup. Detected derived alleles of SNPs M3408, P15, U5 represented subhaplogroup G2a, and L32 represented G2a2b. He was ancestral for downstream M3281, M3423 (representing G2a2b1). Thus, he could be designated as G2a2b (xG2a2b1).

<u>I2394/VEJ9a: C1a2</u>

Derived alleles allow a macro haplogroup CT (M5577, M5584, M5680, M5754), haplogroup C (CTS3223, F2803) and subgroup C1a2 (Y11464) assignment of this individual. Thus, he could be designated as C1a2.

I2783/GEN49: C1a2a

This Hunyadihalom_CA individual gave calls for 41 derived alleles defining haplogroup C. C1 was defined by SNPs F3393, CTS6773. C1a2 subgroup was defined by SNPs M1009, V219, V1535, V1876, V2185, V2722, Y10453, Y10458, Y10471, Y11341, Y11352, Y11390, Y11393, Y11449, Y11456, Y11474, Y11482, Y11590, Z28838. C2a2a was defined by SNPs S23981, Y10462, Y10475, Y11380, Y11493, Z28941. Thus, he could be designated as C1a2a.

I2788/GEN60: G2a2b2a

This Protoboleraz_LCA individual was derived for 56 SNPs characteristic for haplogroup G, 30 SNPs characteristic for G2, and 10 SNPs characteristic for G2a (F4086, L149.1, L31, CTS1879, CTS6026, F2301, CTS6753, F2529, CTS9318, P15). G2a2b was defined by L1259, G2a2b was represented by U8, G2a2b2 by PF3325. Five derived SNPs represented the G2a2b2a subhaplogroup (CTS946, PF3329, CTS4454, CTS10366, Z3243). He was ancestral for Z3220 and CTS12570 defining G2a2b2a1. Thus, he could be designated as G2a2b2a (x G2a2b2a1).

I2789/GEN61: I2c

This Protoboleraz_LCA sample had derived alleles of 29 SNPs (CTS88, CTS646, PF3640, L578, PF3660, PF3661, L758, PF3672, CTS2193, L1197, CTS3517, CTS3641, CTS4088, CTS4209, CTS4273, PF3742, CTS5650, CTS7329, CTS7831, CTS8545, CTS8963, L41, CTS9860, L503, FGC2412, PF3815, M1460, YSC0000272, L847) representing haplogroup I. Derived alleles of SNPs M438 and L68 represented I2, and L596 defined subhaplogroup I2c. Thus, he could be assigned to I2c.

I2791/GEN63: I2c

This Protoboleraz_LCA individual was derived for 53 SNPs characteristic for haplogroup I, M438, L68 SNPs characteristic for haplogroup I2, L597 defining I2c and PF3827 that was formerly characteristic for I2c2, but does not exist on the current ISOGG Y-tree. Furthermore, two derived SNP alleles (L1228-I2a2a2, and P78-I2a2a1b1a) were not supported by several detected ancestral alleles of upstream SNPs on the I2a2-I2a2a branch. Therefore, this individual could be designated as I2c.

I2366/GEN12a: G2a2b2a1a1b1

This well preserved Baden_LCA sample was derived for over 100 SNPs characteristic for haplogroup G, 25 SNPs characteristic for G2 (PF2787, PF2797, PF2800, PF2807, Z3100, M3446, M3480, F1239, F1294, M3491, F1647, PF2909, PF2912, CTS1868, CTS1900, CTS4264, CTS4413, CTS6316, F2319, CTS6692, M3579, F3198, M3607, PF3119, F3536), 15 SNPs characteristic for G2a (F4086, L149.1, L31, CTS6630, CTS6753, F3088, M3397, M3408, Z3506, CTS11463, P15, PF3141). From further downstream SNPs were derived CTS4367 (G2a2), CTS9957 (G2a2b2), seven SNPs characteristic for G2a2b2a (CTS688, CTS946, PF3332, CTS4454, P303, Z3243, Z3481) and three SNPs characteristic for G2a2b2a1 (PF3331, Z3220, PF3337), one characteristic for G2a2b2a1a (PF3346) and three representing G2a2b2a1a1b (PF6850, CTS1899, PF6852). The most downstream derived SNP within the G cluster was Z1815, defining G2a2b2a1a1b1, therefore this individual could be assigned to this subhaplogroup.

I2367/GEN13a: G2a2b2a1a

This well preserved Baden_LCA sample was derived for ca. 80 SNPs representing haplogroup G, 35 SNPs representing G2, 12 SNPs representing G2a, L1259 defining G2a2, F2121 defining G2a2b2. Eleven SNPs were characteristic for G2a2b2a (CTS688, CTS946, PF3329, PF3332, PF3338, PF3339, PF3342, CTS4454, P303, Z3243, Z3481). Further derived downstream SNPs were two SNPs characteristic for G2a2b2a1 (Z3220, PF3337), one characteristic for G2a2b2a1a (PF3346) therefore this individual could be assigned to this most downstream detected subhaplogroup on the Y chromosome tree.

I2368/GEN14a: G2a2b2a1a1c1a

This Baden_LCA sample was derived for two characteristic SNPs of haplogroup G (M3471, M3623), and four subhaplogroup G2 characteristic SNPs (PF2787, F1189, M3488, F1647). Further derived positions downstream on the Y chromosome tree were L190 (G2a2b), CTS688, CTS946, CTS4454 (all three G2a2b2a). The most downstream detected SNPs were Z3236 and Z3440, which belong to the G2a2b2a1a1c1a subhaplogroup, according to the latest ISOGG Y-tree. This individual could be assigned to G2a2b2a1a1c1a.

I2369/GEN15a: G2a2b2a1a1c1a

This well preserved Baden_LCA sample was derived for 80 SNPs characteristic for haplogroup G, seven SNPs characteristic for G2a (L31, CTS1879, F3088, M3401, M3408, Z3506, PF3141), L32 and L30 characteristic for G2a2b. Seven derived SNPs were characteristic for G2a2b2a (CTS688, CTS946, PF3329, PF3332, CTS4454, P303, Z3243). Within this subhaplogroup, PF3345 defined G2a2b2a1a1, CTS342 defined G2a2b2a1a1c and derived alleles of SNPs CTS4472, CTS6763, Z3423, Z3440, CTS11388 defined G2a2b2a1a1c1a. Therefore, this individual could be assigned to the G2a2b2a1a1c1a subhaplogroup.

12371/GEN17a: G2a2a

This Baden_LCA sample was derived for 51 SNPs characteristic for haplogroup G, 11 SNPs characteristic for G2 (PF2807, Z3100, M3465, M3488, F1393, M3493, CTS4264, M3585, F3220, PF3119, F3536), nine SNPs characteristic for G2a (L149.1, CTS1879, F2274, CTS6630, CTS6753, F2529, F3088, P15, PF3141). Six SNPs were defining G2a2a (PF3147, PF3159, PF3161, PF3168, PF3181, PF3185). Further downstream SNPs could not be detected in this sample, therefor this individual could be assigned to subhaplogroup G2a2a.

I2752/GEN21: I2a1

This Baden_LCA individual was derived for 37 SNPs characteristic for haplogroup I. Further derived downstream SNPs were L68 representing I2 and P37.2 defining I2a1. Further derived SNP CTS10100 was characteristic for the subhaplogroup I2a2a1b, but its pair CTS10057 was ancestral, and therefore this subhaplogroup could not be defined. Therefore, this individual could be assigned to the Y-chromosomal I2a1 subhaplogroup.

I2753/GEN22: I2a1a1a

This Baden_LCA individual was derived for 61 SNPs characteristic for haplogroup I, M438 and L68 SNPs characteristic for I2 and P37.2 representing I2a1. Furthermore, derived alleles of M26 represented I2a1a1 and L672 represented I2a1a1a. Within the I2a cluster he was also derived for CTS10057 representing I2a2a1b, however he was also ancestral for five SNPs defining I2a2 (P216, L37, M436, L181, L35) and three SNPs representing I2a2a (S117, S120, M223). Therefore, he could only be assigned to the I2a1a1a subhaplogroup.

I2754/GEN23: I2

This Baden_LCA individual was derived for 17 SNPs characteristic for I (CTS646, L758, Z16985, CTS2536, CTS4209, CTS4273, CTS6231, CTS6265, CTS7469, CTS7502, CTS7831, CTS8420, CTS9264, PF3814, PF3817, CTS11441, CTS11540). He was also derived for subhaplogroup I2 representing SNP L68, but no other downstream SNP could be detected with derived allele. Therefore, this individual could be assigned to the Y-chromosomal subhaplogroup I2.

I2755/GEN24: I

Only 182 SNPs could be called on the Y chromosome of this Baden_LCA individual. Three SNPs with the derived alleles were characteristic for haplogroup I (PF3716, PF3717, PF3836). Ancestral allele calls did not contradict to this haplogroup definition, therefore he could be assigned to haplogroup I.

Prehistoric samples from Germany and Spain:

Samples reported in the Haak et al. 2015 and Mathieson et al. 2015 study, with additional sequence information from newly generated DNA libraries:

I0048/HAL25: G2a2a1

This LNK_EN individual was derived for 39 SNPs characterizing haplogroup G and 16 SNPs representing subhaplogroup G2. Derived alleles for SNPs L31, PF3142, S149, CTS1879, PF2930, F3088, PF3043, P15 order this sample to subgroup G2a and PF3181, PF3185 SNPs further downstream to G2a2a. Derived SNPs Z6201 and Z6042 are characteristic for G2a2a1, but ancestral SNP at Z6679 shows that he was not in the G2a2a1a clade. Therefore, the detected derived alleles assigned this individual to G2a2a1.

He was previously reported as G2a2a [1, 2].

<u>I0056/HAL14: G2a2a</u>

This LBK_EN individual was derived for 27 SNPs defining haplogroup G. Downstream subhaplogroup G2 was represented by four detected SNPs (M3469, F1393, F3220, PF3119) and subhaplogroup G2a by derived alleles of F4086, L149.1, Z3240, F2529, F3088, M3397, Z3506. Terminal SNPs PF3147, PF3175, PF3181 assigned this individual to the G2a2a subhaplogroup.

He was previously reported as G2a2a as well [1, 2].

I0659/HAL2: G2a2a1 (x G2a2a1a)

This LBK individual was derived for 51 SNPs defining haplogroup G, 12 SNPs defining G2, 10 SNPs defining G2a (PF2799, F4086, M3251, PF2863, L31, PF3142, S149, F1975, M3307, PF2969, CTS6026, M3331, PF2991, CTS6630, M3340, PF2997, F2529, M3348, PF3013, M3393, PF3056, P15, PF3112, PF3141). Derived position in FGC7533 represented G2a1, five positions marked G2a2a (PF3151, PF3161, PF3175, PF3184, PF3185). Most downstream detected SNP was PF3170, defining G2a2a1. He was ancestral for PF3177 (G2a2a1a). Therefore, this individual could be designated as G2a2a1 (x G2a2a1a).

He was previously reported as G2a2a1 as well [1, 2].

I0821/HAL24: G2a2a1

This individual was derived for 38 SNPs characterizing haplogroup G. Further 10 derived SNPs defined subhaplogroup G2 (L89, F1239, F1294, F1647, CTS1900, F2319, CTS6742, M3579, M3581, CTS11016), F2274 and F2301 defined subhaplogroup G2a, CTS4367 defined G2a2, and PF3166, PF3175 defined G2a2a. The most downstream detected SNPs were PF3155 and PF3160, designating this individual to subhaplogroup G2a2a1.

He was previously reported as G2a2a1 as well [1, 2].

Revised samples from the Mathieson et al. 2015 study:

10795/KAR6: CT

This LBK_EN individual was formerly determined as T1, however the representing SNP of T1 is only an investigational item (CTS6004), and is not listed on the current version of the Y-tree. The assignment to cluster CT was supported by derived alleles of 13 SNPs. No other haplogroups could be defined by unambiguous SNP calls.

I0551/SALZ3B: G2a2a1

This Salzmuende_MN individual was reported as G2a2a previously. Now, based on the SNP Z6199, he can be further categorized into G2a2a1.

I0581/MIR5 and MIR6: I2a2a

This Iberian Chalcolithic individual was reported as I2a2a1 previously, however it can only be assigned to I2a2a, because one derived (CTS616) and one ancestral (CTS9183) allele was detected, both representing I2a2a1.

Newly reported samples from Germany:

S2014.L2/ HAL15a: G2

This LBK_EN individual was derived for three SNPs characteristic for haplogroup G (M3500, L522, M3564), and F1239 representing subgroup G2. The detected ancestral alleles do not contradict to this haplogroup assignment, thus he could be designated as G2.

S2020.L2/ HAL20b: G2a2a

This LBK_EN individual was derived for five SNPs characteristic for haplogroup G (M3274, CTS2271, CTS5504, PF2983, PF3053), two characteristic for G2 (PF2912, F3226), and one representing G2a (L31). The most downstream SNP that has derived allele was PF3181, defining haplogroup G2a2a, thus he could be designated as G2a2a.

I2021/HAL21a: G2a2a?

This LBK_EN individual was poorly preserved, only fived SNPs showed their derived alleles. Two of them belong to cluster BT, three belong to cluster CT, and two to haplogroup G2a2a (PF3185, PF3181). The detected ancestral alleles do not contradict this haplogroup assignment, but upstream positions would strengthen the definition. Thus he could be designated as an unsure G2a2a.

<u>I2026/HAL27a: G2a</u>

This LBK_EN individual was derived for six SNPs characteristic for haplogroup G (M3464, M3248, M3470, M3472, M3609, L1258), and further two SNPs downstream, representing G2 (M3585), and G2a (U5). He was ancestral for Z6585, characteristic for G2a1a and further downstream SNPs within the G2a1a1 and G2a2a1 clusters. Thus, he could be assigned to haplogroup G2a.

I2037 and S2037.L2/HAL39b: G2a2a1

Library S2037.L2 obtained better resolution for haplogroup G than library L1. This LBK_EN individual was derived for 14 SNPs characteristic for haplogroup G (Page94, L154, L521, L1342, M3450, M3452, M3466, M3473, M3485, M3565, M3574, M3620, PF2815, PF3045). Three SNPs represented subgroup G2 (F1294, M3576, M3626). S2037.L2 gave SNP calls including derived allele of M3342, characteristic for G2a. PF3181 and PF3185 represented G2a2a and PF3155 as most downstream SNP defined subhaplogroup G2a2a1. Thus, he could be designated as G2a2a1.

<u>10802/</u> <u>SALZ77A: IJK (xJ)</u>

This Salzmuende_MN individual was poorly preserved for ancient DNA, and derived Y-chromosomal SNPs could only be detected at SNPs M9121 and M9327 defining BT cluster and at SNP L15, defining IJK cluster. Ancestral allele at the position PF4506 excluded haplogroup J. Therefore, he could be assigned to cluster IJK (xJ).

I1594/Bla28:R1b1a

This poorly preserved Blatterhole_MN sample was derived for four SNPs representing haplogroup P (P230, P237, P240, P244), and for P286 representing haplogroup R1. Further derived downstream SNP L1345 defined R1b1a. Thus, he could be assigned to haplogroup R1b1a.

<u>I1565/Bla8+Bla9+Bla11+Bla24+Bla26(x)+Bla45: I2a1</u>

This Blatterhole_MN individual was derived for 71 SNPs characteristic for haplogroup I. Derived allele of L68 defined subhaplogroup I2, and S238 defined subhaplogroup I2a. Derived allele of the SNP P37.2 represented I2a1. The most downstream derived SNP was detected at position L1286, characterizing I2a1a2a. SNPs representing sub-branches of the I2a1a and I2a1a2 subgroups were not detected. Thus, the assignment to haplogroup I2a1a2a remains unsure, and only the definition of I2a1 is assured.

I1593/Bla16+Bla27+Bla59: R1b1a

This Blatterhole_MN individual was derived for 14 SNP characteristic for haplogroup R (also P227, P280), seven SNPs for R1 (P231, P238, CTS916, CTS2565, CTS3321, CTS5611, L875), and a sole representing SNP for R1b (M343). Subclade R1b1 was defined by SNPs L278, L1349, L822, CTS2229. R1b1a was defined by SNPs CTS7585, FGC35, FGC41, L754, L761. R1b1a1a2 showed both derived and ancestral alleles of characteristic SNPs. Thus, he could only be assigned to haplogroup R1b1a.

<u>I1560_d/ SCHM2a: I</u>

This MN individual from Germany was derived for eight SNPs characteristic for haplogroup I (PF3627.2, FI3, FGC2415, PF3742, CTS6231, CTS10058, PF3800, PF3814). He was ancestral for L64 and L118, characterizing I1 and also for L37 characterizing I2a2 and P220 defining I2a2a. Thus, he could only be assigned to haplogroup I (xI1).

Newly reported samples from Spain:

I2467/Inventario 0/4: I2a2a

This Iberian Chalcolithic individual was derived for five SNPs characterizing haplogroup I (CTS674, FGC2416, L578, FI4, PF3837), furthermore for P221 and P222 characterizing haplogroup I2a2a. Since the detected ancestral alleles on branch I do not contradict to the above listed definitions, he could be assigned to subhaplogroup I2a2.

I2473/ES-6G-110: I2a2a

This poorly preserved Iberian Chalcolithic sample was detected for the derived allele of SNP P123 defining cluster IJ, PF3661 characterizing haplogroup I, and P221 defining I2a2a. Detected ancestral alleles within cluster I were L415 (I2b) and L596 (I2c), S2632 (I2a1b), none of them contradicting with the terminal SNP P221. Thus, he could be designated as I2a2a.

<u>I1975/5.-K18: I2a2</u>

This Iberian Chalcolithic individual was derived for P127 defining cluster IJ, five SNPs defining haplogroup I (PF3641, PF3660, L1197, CTS4088, CTS8876), and L37 defining subhaplogroup I2a2. Since the detected ancestral alleles on branch I do not contradict to the above listed definitions, he could be assigned to subhaplogroup I2a2.

I1976/ES.1/4: I2

This Iberian Chalcolithic individual was derived for eight SNPs defining haplogroup I (PF3641, PF3665, L1197, PF3742, CTS8420, PF3803, PF3829, CTS11979). He was derived for PF3664 defining I2, but ancestral for subgroups I2b (L417). Thus, he could be designated as I2.

<u>I3269 /LY.II.A.10.15066: I2a2a2a</u>

This Iberian Chalcolithic individual was derived for 40 SNPs characterizing haplogroup I, five SNPs defining subhaplogroup I2a2 (P216, P218, L37, L181, L35), and four SNPs defining subhaplogroup I2a2a (L368, P221, P223, P222) and one defining I2a2a2a (L1228). Since the detected ancestral alleles on branch I do not contradict to the above listed definitions, he could be assigned to subhaplogroup I2a2a2a.

I3272 /1.-K11: I2a2

This Iberian Chalcolithic individual was derived for five SNPs (PF3641, FI4, L772, CTS4848, CTS7593) characterizing haplogroup I, and L37 defining subgroup I2a2. He was ancestral to three SNPs defining I1 (S107, L840, S66), and further SNPs within cluster I that are not upstream to I2a2. He could be assigned to subhaplogroup I2a2.

I3276 /LHUE2010.11: G2a2a

This Iberian Chalcolithic individual was derived for 30 SNPs characteristic for haplogroup G, for four SNPs representing G2 (F1294, F1393, CTS6742, F3220), F2529 representing G2a and PF3181, PF3185 characteristic for G2a2a. Detected ancestral alleles within the G branch do not contradict to this definition. Downstream of G2a2a, he was ancestral for Z6134, FGC5671, Z6215, L166, L167 SNPs, characteristic for G2a2a1a2a1a subgroup. He could be assigned to subhaplogroup G2a2a.

I5838/ MIR202-037-n105: I2a2a

This Iberian Chalcolithic individual was derived for 54 SNPs characteristic for haplogroup I, five SNPs defining I2a2 (P218, L35, L37, L181, L368) and P221, P223 characteristic for I2a2a. He could be assigned to subhaplogroup I2a2a.

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Supplementary note 4: Neolithic Anatolians as a surrogate for first European farmers

Previous studies have shown that Neolithic populations from northwestern Anatolia are closely related to the first European farmers (FEF) [1-3]. It is possible though that the Anatolian samples we are using (primarily 6500–6200 BCE from Barcin [1]) are not a perfect surrogate for FEF. We note that Neolithic Anatolia can be modeled as a three-way admixture between Neolithic Iran, Neolithic Levant, and WHG [4]; the affinity to WHG in particular requires care for our analyses. First, if FEF had more or less WHG ancestry than our Neolithic Anatolian sample (i.e., f_4 (Mbuti, WHG; Anatolia, FEF) \neq 0), then we would be over- or underestimating the WHG ancestry proportions in our Neolithic test populations. However, we observe several individuals X, from different populations, with f_4 (Mbuti, WHG; Anatolia, X) \sim 0, but none with f_4 (Mbuti, WHG; Anatolia, X) significantly negative. This indicates to us that in fact f_4 (Mbuti, WHG; Anatolia, FEF) \sim 0. It could also be that f_4 (WHG1, WHG2; Anatolia, FEF) \neq 0 for two different WHG individuals, in which case our inferences of relative affinities to different WHG individuals would be affected. However, to the extent that the farmer ancestry in our Neolithic populations is derived from a homogeneous FEF source, this scenario would not affect the relative affinities of different populations.

To test for differentiation within Anatolia and FEF, we examined the relatedness of different European Neolithic populations to subgroups of Neolithic Anatolians. First, we studied subsets of our 25 samples from Barcin and Mentese [1] according to the three-way admixture model described above. As in ref. [4], we used qpAdm [5] to fit the Anatolians as a mixture of Neolithic Iran, Neolithic Levant, and WHG (the latter represented by Villabruna), but now for each Anatolian sample individually. The inferred proportions varied fairly substantially, but in a mostly continuous fashion that suggested noise rather than true population structure (for example, the Levant component varied from approximately 0-60%, but with estimated standard errors on the order of 20%). For the purposes of this analysis, we created three subgroups consisting of the four individuals with the most and least of the Levant component, plus the 17 in between. Even if these do not represent true sub-populations, we reasoned that we could use them as empirical proxies for hypothetical differentiated Anatolian groups. We chose to divide based on the Levant component because these three subgroups had nearly identical average inferred WHG proportions, and we wished to avoid confounding due to the excess WHG ancestry in European Neoltihic populations. We computed f_4 statistics of the form f_4 (Eur1, Eur2; Anatolia1, Anatolia2) for all pairs of European Neoltihic populations (excluding the three with the most WHG ancestry, namely Blätterhöhle, Iberia MN, and Iberia CA) and any two of the Anatolian subgroups, for a total of 273 statistics. While not all of these are independent (there are effectively between 182 and 273 independent statistics), they show a clear depletion of significant values (Figure S4.1), suggesting that no Neolithic populations are strongly closer to any of the subgroups.

We also repeated this analysis using data from two other populations from Neolithic Anatolia [6]. Instead of the three subgroups defined above, we used our 25 main samples as one group and the Boncuklu and Tepecik-Çiftlik populations from ref. [6] as the other two. Again, we computed all 273 statistics of the form f_4 (Eur1, Eur2; Anatolia1, Anatolia2), and again there is no strong evidence of differential relatedness between any of of the European and Anatolian populations (Figure S4.2). We therefore conclude that the northwestern Anatolian Neolithic samples constitute an appropriate surrogate for FEF.

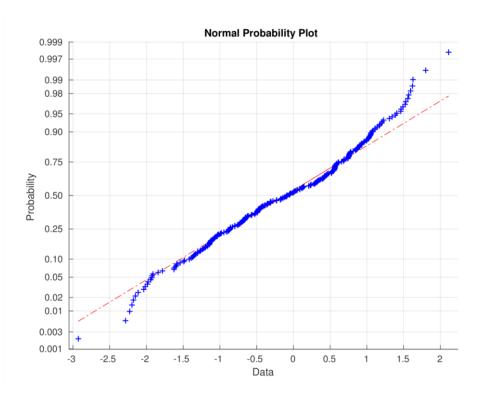


Figure S4.1: Q-Q plot of statistics f_4 (Neolithic1, Neolithic2; Anatolia1, Anatolia2) for all pairs of European Neolithic populations with <20% hunter-gatherer ancestry and the three subgroups of Anatolia Neolithic defined by inferred Levant-related ancestry. Observed values are shown on the x-axis with expected quantiles on the y-axis.

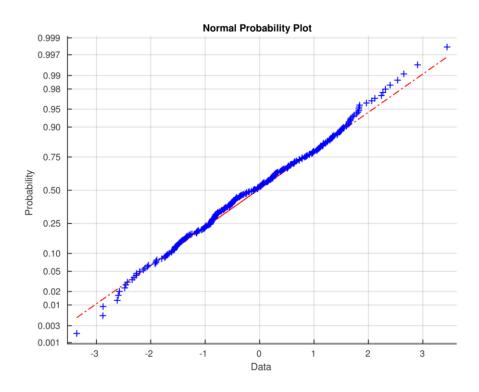


Figure S4.2: Q-Q plot of statistics f_4 (Neolithic1, Neolithic2; Anatolia1, Anatolia2) for all pairs of European Neolithic populations with <20% hunter-gatherer ancestry and the three different pairs of Anatolian Neolithic populations (our main set from Barcın and Mentese, plus Boncuklu and Tepecik-Çiftlik). Observed values are shown on the x-axis with expected quantiles on the y-axis.

References:

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Supplementary note 5: WHG genetic structure and admixture

MDS analysis

For the multidimensional scaling (MDS) analysis of European hunter-gatherers, we combined our four primary WHG individuals – KO1, LB1, LOS, and VIL – with EHG (eastern hunter-gatherers from Karelia and Samara [1, 2]), Bichon (BIC) [3], and El Mirón (ElM) [4]. Previous results suggest that WHG as a group fits into a broad geographic cline of genetic diversity among hunter-gatherers from western Eurasia [1, 5,6], and we wished to determine if substructure within WHG represents a continuation of this trend or if it is organized along different dimensions. We note that we found a PCA analyses of these populations to be heavily influenced by low coverage and missing data. We considered projecting the ancient samples onto PCA axes defined from present-day, high-coverage populations, but because ancient European hunter-gatherers fall outside the variation of present-day western Eurasians, we believe that these axes would not accurately capture the variation among hunter-gatherer groups.

As a starting point for our MDS analysis, we computed a matrix of outgroup f_3 -statistics f_3 (Mbuti; X, Y) for all hunter-gatherer pairs (X, Y), measuring the shared genetic drift between X and Y (Table S5.1). Because we are not interested in population-specific genetic drift, we used a fixed value for all diagonal entries (0.38, slightly larger than any off-diagonal; the results are not sensitive to the exact value). We then subtracted 0.38 from each entry and multiplied all by -1 to yield a matrix in proper distance form. This matrix also has the benefit of being insensitive to differences in sequencing coverage and artificial apparent genetic drift due to pseudo-haploid genotype calls.

After running MDS, we plotted all individuals/populations along the first two dimensions (Figure S5.1). While the positions of the points are differentiated along both axes, they do not fill the space; instead, the variation largely appears to be summarized by a curved line around the origin. This pattern is reminiscent of a well-known behavior of dimensionality reduction techniques (e.g., MDS or PCA) [7, 8] in which a series of observations (including examples of spatial genetic variation data [9, 10]) with linear structure appear in a "horseshoe" pattern when plotted in 2-D. We thus transformed the MDS plot into a single-parameter space (Figure 1D in the main text) by converting all points into polar coordinates and keeping only the radial angle from the origin (measured with zero along the negative y-axis, negative moving clockwise, and positive moving counter-clockwise). EHG is composed of individuals from both Karelia and Samara, and we use the average longitude of the two locations. We note as a caveat that not all of the individuals included here are contemporaneous, but we believe that the observed pattern reveals a legitimate relationship between geography and genetic structure.

Table S5.1: Outgroup f_3 -statistics for pairs of hunter-gatherers

	ElM	EHG	LOS	LB1	BIC	KO1	VIL
ElM		0.268	0.320	0.324	0.314	0.297	0.307
EHG	0.268		0.290	0.288	0.287	0.297	0.286
LOS	0.320	0.290		0.351	0.375	0.349	0.361
LB1	0.324	0.288	0.351		0.345	0.330	0.335
BIC	0.314	0.287	0.375	0.345		0.343	0.357
KO1	0.297	0.297	0.349	0.330	0.343		0.348
VIL	0.307	0.286	0.361	0.335	0.357	0.348	

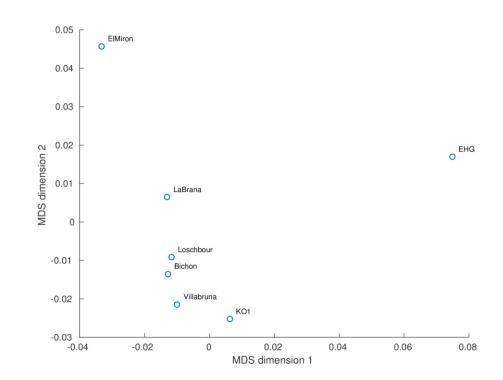


Figure S5.1: First two dimensions of MDS for hunter-gatherer outgroup f_3 -statistics.

Signals of admixture

The three-population test for admixture requires at least two alleles per site in the test population. As a means to accommodate haploid ancient samples, we created pseudo-populations of pairs of WHG individuals and computed f_3 -statistics for all such pairs (from the set of LOS, VIL, BIC, KO1, LB1) as the test population and all possible combinations of two references from among the other individuals in the set, plus EHG. For LOS+BIC, there were no negative statistics; with LOS+VIL, or BIC+VIL, there were one or two that were marginally significantly negative

(-3 < Z < -2); and for pairs including KO1 or LB1, there were a few that were very negative (Z < -6). This leads us to conclude that the significantly negative statistics are due to admixture in KO1 and LB1. (We note that grouping individuals from different time points can produce a spurious negative f_3 signal [P. Skoglund, personal communication], but this effect should not affect our conclusions; BIC and VIL are older than the others, but there are still values Z < -6 for KO1 and Z < -4 for LB1 without using any time-heterogeneous pairs.) We also computed statistics for LOS (with diploid genotypes) alone as the test population and found no negative values (although we note that the lack of a significant test does not prove that it is not admixed).

The signals of admixture in the KO1 and LB1 lineages are consistent with their positions closer to the origin in PCA relative to LOS [1, 2, 11, 12] (Extended Data Figure 1). KO1 shows an affinity to ancient Near Eastern populations as well as to EHG: f_4 (Mbuti, X; Y, KO1) >0 for X = EHG (Z>4), Anatolia (Z>3), Iran, or Natufian, and Y = VIL, LOS, or LB1. Because f_4 (Mbuti, Anatolia; VIL, EHG) <<0, the shared drift between KO1 and Anatolia cannot be explained by EHG-related ancestry and must reflect a separate signal. Using ALDER, we estimated an admixture date of 21.0 ± 7.0 generations (590 ± 195 years) for KO1 (Supplementary Information section 7); combined with the fact that he was found in an EN (Körös) context [13, 14] (although this association is somewhat uncertain), we believe that this relatively recent date likely reflects a small proportion of FEF admixture ($\sim15\%$) in addition to shared ancestry with eastern WHG groups. LB1 meanwhile appears to have ancestry closer to deeper European hunter-gatherers, with, for example, f_4 (LB1, X; Y, Mbuti) >0 (Z-scores between 1.1 and 7.9; Table S5.) for X = VIL or LOS, and Y = EIM, Goyet Q-2 (~15 kya from Belgium [4]), or Goyet Q116-1 (~35 kya from Belgium [4]).

Table S5.2: f_{Δ} -statistics for LB1

Pop X	Pop Y	f ₄ (LB1, X; Y, Mbuti)	Z-score	
VIL	ElM	0.0037	4.8	
VIL	Goyet Q-2	0.0060	7.9	
VIL	Goyet Q116-1	0.0032	4.9	
LOS	ElM	0.0008	1.1	
LOS	Goyet Q-2	0.0024	3.2	
LOS	Goyet Q116-1	0.0011	1.8	

Statistics of the form $f_4(LB1, X; Y, Mbuti)$ for a comparison WHG individual X and older European hunter-gatherer Y, with Z-scores for testing the difference from zero.

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Supplementary note 6: f-statistics and admixture graphs

$f_{\it \Delta}$ -statistics

The simplest tools we use to study hunter-gatherer ancestry in Neolithic populations are f_4 -statistics of the form f_4 (Mbuti, WHG; Anatolia, X) for test individuals or populations X. Under a model of admixture between FEF and WHG, individuals directly descended from FEF with no more hunter-gatherer ancestry than in Neolithic Anatolia are expected to return a value of 0. In the presence of additional hunter-gatherer ancestry, the statistics are expected to be positive, with magnitudes dependent on both the proportion of hunter-gatherer ancestry and the affinity of that ancestry to the individual(s) used to represent WHG. An exception, however, is that if we use an admixed WHG individual, then we can obtain a smaller value even if that individual is closer to the true source [1].

The difference between two such f_4 -statistics computed with different WHG individuals WHG1 and WHG2 is a statistic f_4 (WHG1, WHG2; Anatolia, X) that measures more specifically whether the hunter-gatherer ancestry in X is more closely related to WHG1 or WHG2, although subject to the caveat given above. For the example in Figure 2B in the main text, the pairs of WHG individuals being compared (a western pair of LB1 plus LOS combined as WHG1, and an eastern pair of KO1 plus VIL combined as WHG2) both consist of one individual that is admixed and one that is (approximately) unadmixed, which mostly alleviates the issue mentioned above. We also note that similar trends are observed when using WHG1 = LB1 and WHG2 = KO1 or WHG1 = LOS and WHG2 = VIL. For a more rigorous test of differential WHG relatedness, however, it is necessary to account directly for admixture in the hunter-gatherer source population(s), which we accomplished via our admixture graph modeling.

A possible concern when computing f_4 -statistics using ancient DNA is that certain individuals or populations might show a spurious relationship because of data-related artifacts. Examples could include apparent allele sharing between ancient samples as compared to present-day samples, or between ancient samples with similar processing or sequencing attributes (UDG-treated versus untreated, shotgun versus capture, etc.). These issues can generally be mitigated by avoiding statistics of the form $f_4(X_1, Y_1; X_2, Y_2)$ where the populations X_1/X_2 and Y_1/Y_2 are similar. For our results, we mostly eliminate this possibility by placing our test populations adjacent to Neolithic Anatolians, who share important data properties (ancient samples, 1240k capture, UDG-half). For the few samples with different processing (e.g., CB13, which is shotgun-sequenced and UDG-minus), we did not observe any signs of artifacts. There is relatively more heterogeneity among our WHG reference individuals (especially LOS, although its high coverage may reduce such issues), but again we did not notice any effects on our results (see next section).

Admixture graphs

We aimed to keep our baseline "scaffold" admixture graph (Extended Data Figure 2) as simple as possible while still accurately capturing allele-sharing relationships among the WHG individuals, so as best to isolate our questions of interest regarding hunter-gatherer ancestry in farmers. Thus, our choice of outgroup populations (Mbuti and Kostenki 14) allowed us to disregard certain complexities as extraneous, including differential relatedness to archaic humans and eastern Eurasians.

Our model contains (A) the two outgroups, (B) Neolithic Anatolians (with Basal Eurasian [BE] admixture [2, 3]), and (C) the four WHG individuals KO1, LB1, LOS, and VIL. In accordance with our analyses of WHG structure (Supplementary Information section 5), we found that at least two admixture events involving the WHG individuals were necessary to provide a good fit to the data. Guided by the results in Supplementary Information section 5, we included components of deep hunter-gatherer admixture into LB1 and FEF admixture into KO1, with inferred proportions of 34% and 15%, respectively (the former is not identified with a particular ancestry source, and as a result the proportion may not be well constrained, but this should not affect our results). With LB1 unadmixed, the model contains residual outlier statistics up to |Z| = 9.0 (likelihood score reduced by 110; see below), while with KO1 unadmixed, the model contains residual outlier statistics up to |Z| = 3.3 (likelihood score reduced by 17). Our model is such that we would need one more outgroup in order to solve for the proportion of BE ancestry Anatolians; as specified, we can detect that this admixture event is necessary, but the proportion is confounded with its split point along the non-African lineage. We chose to lock the BE proportion at a reasonable value of 30%, but in fact this does not matter for our model. Our final scaffold, with the inferred best-fitting graph parameters, correctly predicts all empirical f-statistics relating the scaffold populations to within 1.2 standard errors. It is admittedly not a complete historical model, but we believe that it is the simplest well-constrained admixture graph that can accurately explain the observed population relationships. While it is possible that different WHG individuals could be fit as admixed instead, the evidence from Supplementary Information section 5 points to LB1 and KO1 as the admixed pair, and the final scaffold yields an excellent fit to the data.

We built admixture graph models using the qpGraph software [4] with options "outpop: NULL" and "inbreed: YES" and with the full matrix form of the objective function (diag parameter = 0.0001). We restricted to SNPs with at least one allele call in all populations in the graph; our primary scaffold (without a Neolithic test population added) is computed with ~519K SNPs.

When selecting the best-fitting hunter-gatherer ancestry component(s) for Neolithic populations, we use two thresholds based on the model fit score S(G), which is an approximate log-likelihood. First, for comparing single hunter-gatherer sources, we use the fact that the linearity of f-statistics combined with the multivariate normal form of S(G) means that the marginal likelihood of the model as a function of one split point (e.g., the source of hunter-gatherer ancestry) is (generally) approximately normal. Our null hypothesis is that two or more hunter-gatherer sources are

equivalent in their fit, in which case the optimal split point of the hunter-gatherer component would be symmetric to these sources in the tree. Thus, we can view the relative scores with different hunter-gatherer sources as being generated by a normal likelihood under the null; in general, if one source fits best, then models assuming a different source will place the split point at the top of the assigned branch, as close to the optimal source as possible. In order to assign statistical significance, we require a relative score improvement of 2 (equivalent to Z>2); because we are interested in each population separately rather than in the total number of significant values, we do not apply a multiple hypothesis correction.

Second, when deciding whether adding a second hunter-gatherer component significantly improves the fit, we apply a likelihood ratio test, comparing the log-likelihoods of the one-component and two-component models. Under standard assumptions, given that the model with two hunter-gatherer components adds two additional free parameters, two times the difference in scores would be approximately chi-squared distributed with two degrees of freedom; after a threefold Bonferroni correction to account for the three possible additional sources, this translates into a score improvement of \sim 4.1 necessary for the second admixture to be significant at p=0.05. However, for this example, the null case of zero admixture in the second component (which is constrained to be non-negative) falls on the boundary of the parameter space and renders the branch-position parameter non-identifiable, which means that the asymptotic chi-squared approximation may not be valid. For our tests, most of the nominally significant two-component fits were near the threshold (score improvement of \sim 5), so the only population for which we reported two significant components was Iberia CA (whose test statistic was more than four times the critical value according to the chi-squared distribution). Finally, we found that we could not reliably infer the proportions of two different hunter-gatherer components simultaneously, so we only report the presence of multiple components and the total hunter-gatherer ancestry proportion.

To aid in interpretation, we note that in cases where we infer a combination of two hunter-gatherer components, this could represent either two waves of admixture or a single admixture event involving a hunter-gatherer population with mixed ancestry. Likewise, where no single WHG reference individual fits best, this could be due to a source population with no good surrogate in our graph, or a combination of sources, or simply a lack of statistical power (small sample size or low hunter-gatherer proportion).

When adding admixed Neolithic populations to our scaffold (with FEF and WHG ancestry), we placed the possible KO1-related ancestry component in a sister position to the KO1 lineage prior to FEF admixture. We also repeated our analyses using an alternative scaffold in which KO1 and Anatolia share a deeper ancestry component in place of the recent FEF admixture and obtained very similar results. Additionally, we conducted comparisons to several other hunter-gatherer individuals and populations, including lower-coverage late Mesolithic hunter-gatherers, earlier Western European hunter-gatherers, and EHG [5-7]. Using both f_4 -statistics and admixture graphs, we obtained largely concordant results, with no other hunter-gatherers appearing to be

closer references for the hunter-gatherer ancestry in farming populations (although late hunter-gatherers from France and Germany, in particular Ranchot88, are roughly interchangeable with LOS). In order to compare with potential sources more closely related to Eastern European hunter-gatherers, we built an alternative scaffold (Figure S6.1) containing our four primary WHG references plus EHG as a fifth possible source. While it is unlikely that any of our Neolithic populations would have admixture directly from EHG, by allowing for two components of hunter-gatherer ancestry, we would expect to detect signals of potential admixture from a hunter-gatherer source intermediate between WHG and EHG (e.g., Scandinavian hunter-gatherers [5]). Again, we find that the best fits are very similar to those obtained with our primary scaffold model, with little evidence of Eastern European hunter-gatherer ancestry in our Neolithic groups (Extended Data Table 3). As with the primary model, a version of this scaffold without recent FEF admixture in KO1 yielded virtually identical results.

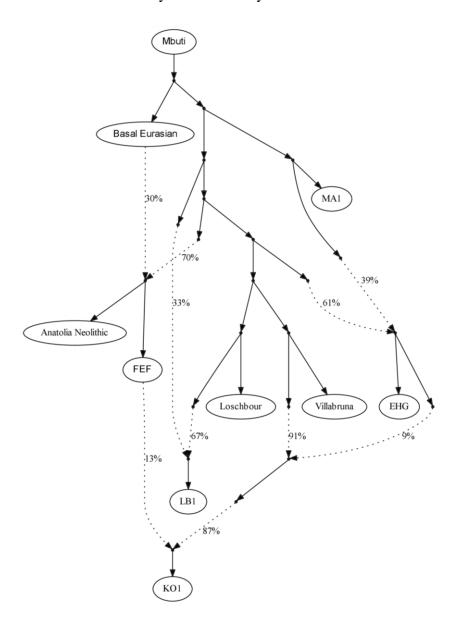


Figure S6.1: Alternative admixture graph scaffold with EHG plus the four WHG reference individuals. Here we also use MA1 as an outgroup in place of Kostenki 14. EHG receives MA1-related admixture, while KO1 has both EHG-related and FEF admixture. The model correctly predicts all empirical *f*-statistics to within 1.4 standard errors.

In order to avoid any bias due to the fact that LOS was shotgun-sequenced, we replicated our primary analyses using capture data instead; we note that these only cover a subset of ~390k SNPs, so that the model using the capture version was fit on the smaller set of SNPs. Generally the results were quite similar, albeit with reduced power due to the lower SNP count. We were most interested in the populations for which we had inferred LOS-related components in the primary analysis: for Blätterhöhle and Iberia MN, the inferred components remained the same (with LOS-related ancestry improving the fit but not quite significantly), while for Germany MN, the model lost power to distinguish between the alternative sources. We thus concluded that the original results were robust. We note that for Iberia CA, the graph with capture LOS data fit best with LB1-related ancestry plus a component related to any of the other three WHG individuals, whereas LOS did not fit as well in the primary analysis; to be conservative, we adopted the more inclusive combination from the capture version in reporting our main results.

Mixture proportions

We used two different methods to estimate hunter-gatherer ancestry proportions in Neolithic farmers. First, as discussed above, the statistic f_4 (Mbuti, WHG; Anatolia, X) reflects both the mixture proportions of X and the proximity of the reference WHG to the true mixing hunter-gatherer population. We reasoned that if we pooled together our four WHG reference individuals into a composite WHG population, then the resulting f_4 -statistic values should be less sensitive to differential WHG affinity. Second, we can use the inferred best-fitting mixture proportions from our admixture graph analyses. Here, in principle, results obtained with different WHG sources could show a slight directional bias, but we observe for our test populations that the effect is minimal (at most 1–2%). Thus, for this approach, we used the proportions inferred in the model with the best-fitting choice(s) of WHG source for each Neolithic population.

Empirically, we find that these two sets of mixture proportion estimates are extremely well correlated at the population level (i.e., using a full population grouping for X; r^2 =0.99 for linear regression, with an intercept of 0.1%; Figure S6.2). If we omit Blätterhöhle, the regression line is very similar (r^2 =0.98), and likewise if we constrain the intercept to be zero (r^2 =0.99). In light of this close proportional relationship, we consider the two measurements to be essentially interchangeable. In particular, where we report population-level mixture proportions, we use the direct estimates from the admixture graphs, whereas for individual-level results, we use f_4 -statistic values scaled by the best-fitting zero-intercept linear regression line (virtually indistinguishable from Figure S6.2). The one exception is that for population-level standard errors, we also used the

more straightforward f_4 -statistic-based values, again scaled by the same factor (which we additionally found were more conservative than admixture graph-inferred standard errors in a few empirical tests).

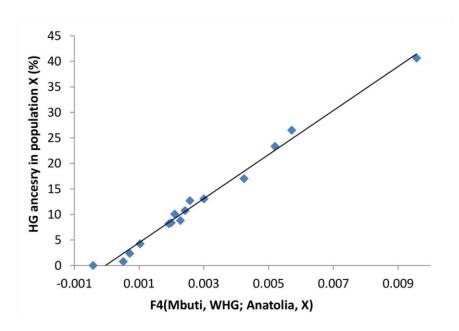


Figure S6.2: Correlation between f_4 (Mbuti, WHG; Anatolia, X) and admixture-graph-inferred hunter-gatherer ancestry proportions for Neolithic population groups X (best-fit linear regression line shown).

Intra-population heterogeneity in hunter-gatherer ancestry among our Neolithic samples is generally low. Of the 15 populations, 10 have empirical standard deviations of hunter-gatherer ancestry less than 3%, which is comparable to the uncertainty in the estimates as assessed by block jackknife (approximately 2% for most individuals and higher for those with lower coverage). Three have standard deviations of 3.1–3.5%: Starčevo, TDLN, and Iberia CA. Only Germany MN and Blätterhöhle are higher (5.3% and 6.6%, respectively), and most or all of the excess variance is caused by a single individual (the Rössen individual Hal13a and the more recent individual Bla28, respectively). Some degree of heterogeneity is expected for recent admixture events even in an unstructured, randomly-mating population [8], although for our populations this contribution (on the order of 0.5%, except slightly higher in Blätterhöhle) is smaller than our statistical uncertainty.

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Supplementary note 7: ALDER

We estimated dates of admixture using ALDER [1], which takes advantage of linkage disequilibrium (LD)—i.e., correlation between nearby alleles—caused by non-recombined blocks of recently admixed ancestry along chromosomes. Our assumed model is that the genomes of Neolithic individuals consist of stretches of ultimately FEF and WHG ancestry. In order to maximize the resulting LD signal, we use Neolithic Anatolians (our surrogate for FEF) and WHG as reference populations when computing weighted LD (see below). A test population could still have a complicated history under this model, for example multiple waves of hunter-gatherer admixture, continuous admixture, admixture between farming groups with different proportions of hunter-gatherer ancestry, or admixture with hunter-gatherers already harboring FEF ancecstry. All of these would be expected to produce admixture LD, with the final inferred date being an average over the admixture process. Notably, under all of these scenarios, the optimal reference populations for weighting remain the ancestral FEF and WHG. These weights will also pick up LD signal from other admixture sources, for example from the steppe, but our sampled populations very likely only possess FEF and WHG ancestry. We also note that while ALDER can use LD patterns to determine whether or not a test population is confidently admixed, here we assume our FEF+WHG model from the beginning and do not make use of the ALDER admixture test results.

In this work, we introduce a new procedure that yields individual-level ALDER results. There are four main motivations for this scheme. First, of course, it means that we can study admixture histories of single individuals. Second, the ALDER model assumes that the test population is sampled at a single time point, with the admixture event at the same time in the past for all individuals. In this study, on the other hand, we are often grouping together individuals who lived several generations apart, so that even assuming they are descended from the same admixture event, their LD curves will have different decay rates. Pooling such individuals together would thus make the interpretation of the LD signal more difficult, and we are more comfortable as a result estimating calendar dates of admixture for each individual first and then averaging these together. Third, our procedure alleviates the impact of low-coverage DNA sequences for ancient individuals: for the main ALDER algorithm, all SNP pairs that are both missing in any test individual are discarded, leading to compound SNP pair loss when running with a population of any appreciable size. Finally, our procedure makes it possible to include all individuals a priori, without having to pre-select the ones that have strong LD signal. Those with low coverage or that have noisy LD signal for any reason will either fail to fit or will (presumably) have noisy date estimates, which are then down-weighted, as would be desired (see below).

Ordinarily, it is not possible to obtain a date of admixture for a single individual using *ALDER* because at least two observations are necessary in order to compute covariances between loci. (If one has phased genotype data available, then a pair of chromosomes from a single individual are sufficient to compute LD, but for unphased data two individuals are required.) We thus take advantage of the fact that European Neolithic populations can be modeled as mixtures of FEF and

WHG ancestry and the availability of close surrogates for FEF by creating "pseudo-populations" consisting of one European test individual and one Anatolian Neolithic individual. For such a pair, using the proper references, the observed weighted LD signal should be due exclusively to the admixture in the test individual: the unadmixed Anatolian Neolithic "helper" individual will make the signal noisier but will not contribute to the covariance between loci resulting from FEF and WHG ancestry blocks (see below for further discussion).

In our procedure, we group each test individual with five different Anatolian Neolithic individuals (chosen for their good performance in a series of pre-tests) and run *ALDER* for those five pairs with Anatolians (the other 24 individuals) and WHG (KO1, LB1, LOS, VIL, and BIC) as the two references. If any one of those five runs fails to fit (imposing an upper limit of 500 generations and a lower limit of 1 generation for each jackknife replicate, leaving out one chromosome at a time), then we consider that test individual to have failed. If all five give a finite result (mean and standard error), then we use a jackknife procedure to average them together, weighting by the inverse of the (jackknife-inferred) standard error. This yields the final date estimate for each individual. To aggregate for a full population, we convert the individual *ALDER* dates into calendar dates (combining the standard errors on the admixture date and the sample age; see below) and then again use a jackknife procedure to average the calendar dates, this time weighting by the inverse of the full standard error times the *ALDER* date (see Figure S7.1).

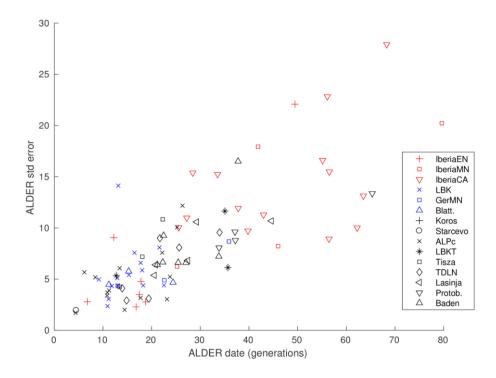


Figure S7.1: *ALDER* date plotted against jackknife-inferred standard error for each Neolithic individual. The errors appear to be strongly correlated with the magnitude of the date, which motivates the use of *Z*-score weighting for population averages.

We found that this grouping procedure was more effective when using a paired individual from the majority ancestral population (i.e., using Anatolians rather than WHG for European Neolithic test individuals with FEF ancestry > 0.5). In three cases, namely the Blätterhöhle individuals Bla8 and Bla28 and the hunter-gatherer KO1, we used WHG individuals (the standard four, plus BIC, minus KO1 for its own test) instead of Neolithic Anatolians in the pairs. We considered the possibility that admixture in KO1 could influence the results for Bla8 and Bla28, but the inferred dates were virtually unchanged, so we used the KO1 helper pair together with the other four. For the new hunter-gatherer individual TIDO2a from Hungary, the Anatolian helper individuals unexpectedly yielded a finite *ALDER* result whereas the WHG helpers did not, so that is the date we report.

Where possible, we also compared our primary, individual-based date estimates to *ALDER* results obtained by running on full population groups (Extended Data Table 4). In order to make the different values equivalent, we converted the individual-based estimates to numbers of generations in the past by subtracting the individuals' average ages (weighted by the same factors) from the population's average calendar date of admixture. The two sets of estimates are generally very similar and always statistically consistent, with the exception of Protoboleráz. In most cases, the individual-based dates have lower standard errors, with slightly higher point estimates on average.

For curve-fitting, we use the genetic distance starting point recommended by the program. In theory, the individual-level *ALDER* technique could be more susceptible to confounding from background LD (potentially yielding inflated date estimates) because the test-population pairs have relatively low power to detect correlated LD (which determines the low-distance threshold). However, we see at most minor evidence of such an issue, with the individual-based dates only slightly higher on average than the full-group *ALDER* results. Additionally, because we use inverse-standard-error weighting over the five paired runs to estimate individuals' admixture dates, such that pairs with lower values will tend to be given more weight, any background-LD bias may be somewhat compensated. We also note that for group-level estimates with individuals from different time points, one might expect the samples with lower *ALDER* dates to have a disproportionate amount of LD signal (due to les time for recombination to have eroded LD) and thus cause a downward bias.

Another possible concern could be that the FEF population or the "helper" Anatolian individuals in particular could have some admixture LD, or that by using Anatolians as one reference, some correlated non-admixture LD could be biasing the signal (although in theory this should be accounted for by the genetic distance threshold). As an additional test, though, we ran all pairs of the five Anatolian helper individuals through the same *ALDER* pipeline (i.e., treating each one in turn as the test individual). If there were some admixture in Anatolians that could affect our results for Neolithic test individuals, then by using the same WHG+Anatolia references, we should find such a signal here. In fact, though, for each Anatolian individual, there is at least one pairing that fails to fit (i.e., all five fail under our fitting criterion), plus none of the groupings has both decay and curve amplitude significantly different from zero. Thus, there appears to be minimal confounding LD signal in Neolithic Anatolia.

While in some cases it can be possible to separate different admixture events using weighted LD patterns [2-4], for the examples here, especially with ancient DNA, we do not have sufficient power. In all cases, however, the inferred dates can be used as bounds, indicating the latest that mixture could have started and the earliest that it could have ended. We also note that admixture LD can be generated through mixture between two populations that are already admixed, either farmers with different proportions of hunter-gatherer ancestry or mixture between farmers and farmer-admixed hunter gatherers. Thus, while admixture estimates for earlier Neolithic groups can most likely be interpreted directly as dates of admixture between farmers and hunter-gatherers, we view later dates as more general evidence of population discontinuity involving differential hunter-gatherer ancestry.

References:

- [1] Loh, P.-R. *et al.* Inferring admixture histories of human populations using linkage disequilibrium. *Genetics* **193**, 1233–1254 (2013).
- [2] Moorjani, P. *et al.* Genetic evidence for recent population mixture in India. *Am. J. Hum. Genet.* **93**, 422–438 (2013).
- [3] Hellenthal, G. et al. A genetic atlas of human admixture history. Science 343, 747–751 (2014).
- [4] Pickrell, J. K. *et al.* Ancient west Eurasian ancestry in southern and eastern Africa. *Proc. Natl. Acad. Sci. U. S. A.* **111**, 2632–2637 (2014).

Supplementary note 8: Blätterhöhle sample overlap

Here we describe in more detail our assignment of the 17 Blätterhöhle samples to a set of four distinct individuals. Our primary tool is a genetic analysis based on the rate of allele sharing between unrelated and related individuals (an approach adopted independently in the software READ [Error! Reference source not found.]). For a given pair of samples, we choose one read at random from each sample at each SNP and define a mismatch rate as the proportion of SNPs (covered at least once by both) at which the two reads carry different alleles. Any pair of samples derived from two unrelated individuals in a population will tend to have similar mismatch rates, while overlapping samples from the same individual will have a mismatch rate half as high (50% of comparisons will be from the same chromosome and hence will always match, and the other 50% will be across the individual's different chromosomes); close relatives will be intermediate.

In Figure S8.1, we show the empirical mismatch rates for all pairwise comparisons of Blätterhöhle samples. The observed pattern precisely matches the expectation in the presence of duplicate samples: the majority of comparisons lie in a cluster at approximately 0.24, defining the unrelated mismatch level, while the others fall at approximately 0.12. Moreover, the lower cluster is fully transitive, i.e., if the pairs A-B and A-C are both in the lower cluster, then B-C is as well. We also confirmed that when we sample twice from the same library, so that the pair are surely from the same individual, we also observe mismatch rates of ~0.12 (not shown). These results thus define the sets of overlapping samples: Bla5/Bla7/Bla13/Bla26(o)/Bla30/Bla54, Bla8/Bla9/Bla11/Bla24/Bla26(x)/Bla45/Bla49, Bla16/Bla27/Bla59, and Bla28 (the last individual being represented by only a single sample).

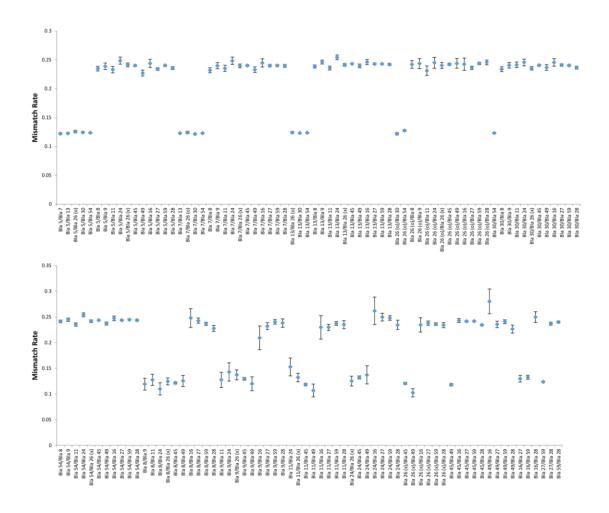


Figure S8.1: Average allele mismatch rates across all SNPs for each pair of Blätterhöhle samples. Values of \sim 0.24 correspond to pairs of samples from different individuals, while values of \sim 0.12 correspond to pairs of samples from the same individual. Bars indicate one standard error in each direction (as assessed by block jackknife).

We also confirmed that population genetic inferences are consistent when we analyzed the 17 samples separately. Both hunter-gatherer ancestry proportions and dates of admixture (when possible to estimate) show very good agreement within the groups of samples assigned to the same individual (Table S8.1). Bla16 and Bla27 were previously assigned to different mtDNA haplogroups (H11a and U5b2a2, respectively), but in our analyses they were both typed as U5b2a2 (and indeed no overlapping samples were found to have different uniparental markers). Stable isotope ratios [2] are likewise consistent: with the exception of Bla26(x), samples are inferred to overlap with Bla8 (the hunter-gatherer individual) if and only if they display isotope profiles associated with a hunter-gatherer-fisher lifestyle.

Table S8.1: Ancestry proportions and dates of admixture for Blätterhöhle samples

Sample name	Hunter-gatherer ancestry (%)	ALDER date (generations)
Bla5	39.7 ± 2.2	24.8 ± 6.3
Bla7	40.9 ± 2.3	23.5 ± 5.9
Bla13	41.5 ± 2.0	22.4 ± 5.1
Bla26(o)	41.2 ± 2.9	
Bla30	41.9 ± 2.2	33 ± 8.8
Bla54	41.7 ± 2.0	23.6 ± 5.4
Bla8	76.7 ± 4.2	
Bla9	61.2 ± 5.6	
Bla11	64.8 ± 5.2	
Bla24	59.3 ± 6.1	
Bla26(x)	66.9 ± 3.6	8.2 ± 1.6
Bla45	73.8 ± 2.0	11.3 ± 2.6
Bla49	78.9 ± 4.8	14.6 ± 3.9
Bla16	30.2 ± 6.7	
Bla27	41.0 ± 2.3	
Bla59	39.5 ± 2.0	15.2 ± 4.9
Bla28	53.0 ± 2.7	11.3 ± 4.3

Results of population genetic analyses for Blätterhöhle samples. Samples corresponding to the same individual are grouped between horizontal lines. Values are shown plus or minus one standard error.

In a few places, however, these findings conflict with prior results and expectations [2]. First, it is surprising that Bla26(o) and Bla26(x), nominally from the same bone fragment, are inferred to originate from distinct individuals. Second, while the majority of the radiocarbon dates for overlapping samples are consistent, a few confidence intervals are disjoint (Table S8.2). We believe that the disagreement between Bla26(o) and Bla26(x) is almost certainly due to mislabeling at some point in the sample processing pipeline, as the two samples are genetically highly differentiated from each other and also highly concordant with their overlapping samples as assigned. This would also explain the apparently inconsistent radiocarbon date and stable isotope ratios for Bla26(x) (and potentially Bla26(o)). Given this observation of mislabeling, it is possible that other instances also occurred and could be the reason for the most significant date outliers, while minor disagreements on the order of tens of years could perhaps simply be due to statistical noise. Other possibilities could include minor contamination or discrepancies in preservation. We note that for simplicity, despite these findings, we retained the original labels for our genetic results.

Table S8.2: Radiocarbon dates for Blätterhöhle samples

Sample name	Radiocarbon date (BP)	Calibrated date (BCE)
Bla5	4580 ± 30	3498–3117
Bla7	4860 ± 30	3704–3539
Bla13	4730 ± 25	3634–3378
Bla26(o)	4510 ± 35	3354–3097
Bla8	4950 ± 30	3786–3657
Bla9	4905 ± 25	3713–3642
Bla11	5145 ± 30	4038–3810
Bla24	4845 ± 35	3703–3532
Bla26(x)	4510 ± 35	3354–3097
Bla16	4615 ± 30	3512–3344
Bla27	5055 ± 35	3958–3773
Bla28	4465 ± 30	3337–3024

Available radiocarbon dates (uncalibrated and calibrated, the former plus or minus one standard error and the latter 95% confidence intervals) for Blätterhöhle samples. Samples corresponding to the same individual are grouped between horizontal lines. For our analyses in the main text, we create conservative individual-level date intervals by taking the union of the intervals for all corresponding samples other than Bla26.

Finally, we also note that we make an exception and include individual Bla5 in our main analyses despite the fact that it has evidence of mitochondrial contamination (~6–25% per library). There are two primary reasons why we treat this individual as a special case. First, it is differentiated from all other samples with signs of mtDNA contamination by its substantially higher coverage on the nuclear genome—approximately 5x for Bla5 as compared to < 1x for all others (median ~0.1x; Supplementary Table 1)—indicative of higher-quality data. Second, because it is comprised of data from six merged samples, we have the ability to compare the results of population genetic analyses across multiple replicates from independent bone and library preparations (and with varying estimated levels of mtDNA contamination). As shown in Table S8.1, our inferences are in excellent agreement among the Bla5, Bla7, Bla13, Bla26(o), Bla30, and Bla54 samples, and our results for Bla5 also do not deviate substantially from the other Blätterhöhle individuals. As a result, while acknowledging the possibility of contamination, we believe that Bla5 is sufficiently robust and accurate in our analyses to warrant its inclusion.

References:

- [1] Kuhn, J. M. M., Jakobsson, M. & Günther, T. Estimating genetic kin relationships in prehistoric populations. *bioRxiv* preprint 100297 (2017).
- [2] Bollongino, R. *et al.* 2000 years of parallel societies in Stone Age Central Europe. *Science* 342, 479–481 (2013).

Supplementary note 9: Simulations

We used the Markovian coalescent simulator MaCS [1] to generate simulated data for a variety of temporal admixture scenarios. In all cases, we specified ancestral populations A and B diverging 1200 generations in the past and an admixed test population C splitting from A n generations in the past (where n=10,20,...,100) and then receiving gene flow from B. For simplicity, we created different simulations varying the value of n instead of using populations sampled at different time points. For a given admixture scenario, the time course was always the same from the split point (time 0) to the present (time n), so that the different simulations yielded data for different times since the onset of mixture. The slight changes in the branch lengths for populations A and B should be negligible for our purposes. We set the effective population sizes to 20,000, 10,000, and 20,000 for A, B, and C and simulated 20, 20, and 40 diploid individuals, each with 10 chromosomes of length 100 Mb; we set the mutation and recombination rates to be 1.0×10^{-9} and 1.25×10^{-8} per base per generation, respectively (the mutation rate is artificially low so as to keep the number of SNPs to a reasonable total).

In all cases, we specified time courses of admixture so that population C would contain a total of 80% ancestry from population A and 20% from population B after 100 generations (meaning that in scenarious involving ongoing mixture, C would have less than 20% B-related ancestry when sampling at n<100). For ALDER dates as a function of time since initial contact, only the relative admixture rates each generation should have an effect and not the total proportion, at least for values in our typical range. We simulated two categories of admixture scenarios, as follows:

- 1. Continuous admixture. First, we simulated uniform continuous admixture each generation, in the sense of a constant rate of gene flow (so that the total mixture proportion in C increases slightly sub-linearly as a function of time due to dilution). We also created histories in which the the rate of gene flow decreases exponentially over time (as though a fixed proportion of population B were being absorbed each generation without replacement), by either 1%, 3%, or 5% each generation. We did not simulate scenarios with increasing gene flow over time because we observed that they would be a poor fit to the data (below the bottom yellow curve in Figure 3 and Extended Data Figure 5 in the main text).
- 2. Pulse-plus-continuous admixture. Here we simulated an initial pulse at time 0 followed by uniform continuous admixture, with the pulse contributing either 5%, 10%, 15%, or 20% B-related ancestry (i.e., 1/4, 1/2, 3/4, or all of the hunter-gatherer ancestry found in C by the end of the time series) and the continuous portion contributing a total of 15%, 10%, 5%, or 0% B-related ancestry.

When running *ALDER*, we used the full simulated population groups—C as the test population and A and B as references—fixing the fitting start point at 0.5 cM. We carried out the procedure for 10 independent simulations and recorded the mean of the final inferred date over the 10 runs. For

display in Figure 3 and Extended Data Figure 5, we fit smooth functions through the resulting data points (Figure S9.1).

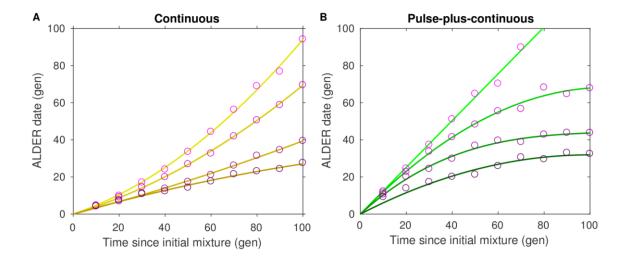


Figure S9.1: Admixture dates as a function of time for simulated data. Circles show observed average *ALDER* dates, with smooth curves as used in Figure 3 and Extended Data Figure 5 in the main text. (A) Continuous admixture simulations: from top to bottom, diminishing 5% per generation, diminishing 3%, diminishing 1%, and uniform. (B) Pulse-plus-continuous admixture simulations: from top to bottom, all hunter-gatherer ancestry in a pulse at time zero; 3/4 of final hunter-gatherer ancestry in an initial pulse, followed by uniform continuous gene flow; 1/2 in initial pulse and the rest continuous; and 1/4 in initial pulse.

References:

[1] Chen, G., Marjoram, P. & Wall, J. Fast and flexible simulation of DNA sequence data. *Genome Res.* **19**, 136–142 (2009).